ECOLOGY AND PHYSIOLOGY OF SELECTED MANGROVE SPECIES UNDER ELEVATED SEA LEVEL

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ABSTRAK

Peubahan iklim seperti kenaikan aras air laut dan karbon dioksida di atmosfera dijangka mempengaruhi komuniti tumbuhan pada skala yang berbeza. Eksistem hutan paya bakau sangat diancam oleh kenaikan aras air laut kerana berada berada d bawah aras laut pada jangkamasa lebih panjang. Empat set eksperimen dilakukan di rumah kaca untuk mengkaji ekologi dan fisiologi *Avicennia alba, Avicennia marina* dan *Bruguiera parviflora* di bawah simulasi kenaikan aras air laut. Keputusan menunjukkan di bawah kenaikan aras air, *A. alba* dan *B. parviflora* menunjukkan tindakbalas yang serupa iaitu penambahan ketinggian pada awal kajian, pengurangan diameter batang dan pembentukan daun, serta penambahan keguguran daun. Jumlah biojisim dan kadar tumbesaran relatif (RGR) juga turut menurun. Begitu juga, parameter fisiologi iaitu asimilasi, konduktan stomata dan sekuestrasi karbon turut menurun. Sebaliknya, *A. marina* menunjukkan penambahan ketinggian sepanjang eksperimen tetapi menunjukkan pengurangan diameter batang di bawah kenaikan aras air. Pembentukan daun dan keguguran daun tidak bertindakbalas terhadap kenaikan aras air. Begitu juga, biojisim dan RGR bagi *A. marina* tidak dipengaruhi oleh kenaikan aras air. Parameter fisiologi dan karbon sekuestrasi bagi *A. marina* kurang bertindakbalas terhadap kenaikan aras air berbanding dengan *A. alba* dan *B. parviflora*. Selain itu, kenaikan aras air mempengaruhi ciri-ciri karakter tanah. Keupayaan redox dan pH tanah adalah berkurang dengan bererti bagi *A. alba* dan *B. parviflora*. Tumbesaran dan tindakbalas fisiologi bagi ketiga spesis menunjukkan *A. alba* dan *B. parviflora* adalah lebih sensitif terhadap kenaikan aras air laut, sementara *A. marina* lebih senang beradaptasi. Keputusan ini sejajar dengan taburan ketiga-tiga spesis sepanjang zon pasang surut di mana *A. marina* hidup berdekatan dengan air laut sementara *A. alba* dan *B. parviflora* lebih terjurus di kawasan berdekatan daratan.

ABSTRACT

Climate change components such as sea level rise and increased atmospheric carbon dioxide is likely to affect plant communities at various scales. Mangrove ecosystems are highly threatened by the sea level rise as a result of prolonged waterlogging. Four sets of experiments were set up to study the ecology and physiology of *Avicennia alba*, *Avicennia marina* and *Bruguiera parviflora* under simulated sea level rise. In elevated sea level, *A. alba* and *B. parviflora* indicated similar responses with initial height increments, decreased stem diameter and leaf formation; and increased leaf fall. The total biomass and relative growth rates (RGR) also decreased. Similarly, physiological parameters such as assimilation, stomatal conductance and carbon sequestration decreased. In contrast, *A. marina* had stem height increment through out the study period but had decreased diameter increment under elevated sea levels. Leaf formation and leaf fall was insensitive to tidal flooding. Likewise, biomass and RGR of *A. marina* remained unaffected by the tidal treatments. Physiological parameters and carbon sequestration of *A. marina* was affected to a lesser extent than *A. alba* and *B. parviflora*. In addition, elevated sea level affected soil physical characteristics. The differences varied significantly between species where soil redox potential and pH was highly reduced in *A. alba* and *B. parviflora*. The growth and physiological responses of these three species indicate that *A. alba* and *B. parviflora* are more sensitive to sea level rise, while *A. marina* is better adapted to the associated stresses. These results correspond with the distribution of these three species along the intertidal zone where *A. marina* is found at the lower intertidal zones, while *A. alba* and *B. parviflora* occupy the landward margins of the mangrove forests.

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µ	Micro
A	Assimilation
A_{400}	Photosynthesis at ambience
ABA	Abscisic Acid
A_{max}	Maximum Photosynthesis
ANOVA	Analysis of Variance
C_a	Atmospheric Carbon dioxide Concentration
cm	Centimetre
CO_2	Carbon dioxide
df	Degree of Freedom
E	Transpiration
Eh	Redox Potential
F	F-Statistics
g	Gram
g_m	Carboxylation Efficiency
g_s	Stomatal Conductance
IPCC	Intergovernmental Panel on Climate Change
LI	Lower Intertidal
m	Meter
m^{-2}	Per Meter Square
mg	Milligram
mm	Millimetre
mol	Mole
MS	Mean Square
MSL	Mean Sea Level
mv	Millivolt
n	Sample Size
p	Probability
PAR	Photosynthetically Active Radiation

pH	Measure of the acidity or alkalinity
ppm	Parts per Million
PSD	Particle Size Distribution
PWUE	Photosynthetic Water User Efficiency
r	Coefficient of Correlation
RGR	Relative Growth Rate
ROL	Radial Oxygen Loss
s	Second
s^{-1}	Per Second
SPSS	Statistical Package for the Social Sciences
UI	Upper Intertidal
USA	United States of America
USD	United States Dollar
Γ_{CO_2}	Carbon dioxide Compensation Point

CHAPTER 1 - INTRODUCTION

1.1 INTRODUCTION

Numerous studies over the last decades provide ample evidence on rapid climate change, predominantly facilitated by the anthropogenic activities (Mastrandrea and Schneider, 2001; Mitchell, 2002). These rapid changes in the environment may affect a wide variety of organisms (Edwards and Richardson, 2004; Thomas et al., 2004) and several ecological processes have shown responses to minor climate changes that have occurred in the past century (Parmesan and Yohe, 2003). Model simulations on climate change effects on ecosystem dynamics have been developed to assess the ecological responses of ecosystems in a changing environment (Easterling et al., 2000; Pearson and Dawson, 2003). Long term studies on physiology, distribution, and phenology of some species show results consistent with theoretical predictions indicating that climate change components are already affecting a variety of organisms at various scales (Perry et al., 2005; Thuiller et al., 2005; Warren et al., 2001). However, the magnitude of the effects of climate change on the living organisms is highly uncertain and much debated (Lomborg, 2001).

One of the remarkable ecosystems that straddle between the sea and the land is the mangrove ecosystems or mangals (Field et al., 1998a). Mangroves are a taxonomically diverse group of woody plants that occupy estuaries and intertidal zones throughout the tropics and subtropics (Ellison and Stoddart, 1991; Field et al., 1998b). Mangrove ecosystems sustain a comparatively low diversity of dominant higher plants that show high fidelity to particular habitats (Woodroffe, 1987). These plants possess a number of

specialized physiological and morphological adaptations that enable them to colonize the interface between the sea and the land (Ball, 1988b; Kathiresan and Bingham, 2001).

The multi-facet roles of these ecosystems have been highly credited among the scientists but the same view has been accepted by the broader community only recently. The remarkable values of these forests from a scientific standpoint can be divided into ecological, community and economic values. From an ecological perspective, mangrove forests are among the most productive ecosystems (Komiyama et al., 2008). Studies on mangrove productivity suggest that mangroves are capable of sequestering 1.5 tonnes of carbon per hectare per year (Gong and Ong, 1990). High productivity plays a crucial role in maintaining the health and function of the marine food chain (Kristensen et al., 2008). As the residents of the interface between the sea and the land, mangrove forests provide habitats for a variety of marine and terrestrial organisms (Nagelkerken et al., 2008). Apart from true mangrove species, mangrove forests support several other plants such as palms, shrubs and ferns. The lower parts of these forests show a characteristic feature of intermingled, highly specialised root systems of the mangrove plants. A number of marine organisms, especially the sessile ones and juvenile fish, take refuge in these specialised root systems as these provide both nutrients and protection (Laegdsgaard and Johnson, 2001). Similarly, the aerial canopy provides breeding areas and habitats for a number of bird species (Nagelkerken et al., 2008; Zou et al., 2008).

The mangrove forests play a fundamental role in human sustainability and livelihoods (Alongi, 2002) especially in the developing countries of the tropics where a large proportion of the population exists near the coasts. Such populations are characterized by relative geographical isolation, chronic poverty and substantial reliance on the products of marine and coastal resources for their livelihood (Kunstadter et al., 1986).

Mangrove ecosystems are heavily utilized for food, medicine and mangrove wood is extensively used for fuel and construction (Walters et al., 2008). Furthermore, mangroves provide recreational grounds for these communities.

The annual economic value of mangroves have been estimated to be USD 200,000-900,000 ha^{-1} based on the cost of the products and services they provide (Wells et al., 2006). In Malaysia, the value of mangrove forests in just storm protection and flood control is estimated at USD 300,000 km^{-1} while the value of the mangroves of Moreton Bay, Australia is USD 4850 ha^{-1} based only on the catch of marketable fish (RAMSAR Secretariat, 2001). Similarly, the economic value of mangrove restoration range between USD 225-216,000 ha^{-1} (Lewis, 2005).

Despite the socio-economic values of mangrove forests, 35% of the total area of the mangrove forests has been lost in the last two decades, losses that exceed those for tropical rain forests and coral reefs (Valiela et al., 2001). Reduced mangrove area and health pose threats to human safety and shoreline development from erosion, flooding, storm waves and surges, and tsunami (Kathiresan and Rajendran, 2005). Mangrove loss will also affect coastal water quality, biodiversity; and destroy juvenile fish and crustacean nursery habitat. Similarly, reduced mangrove area will affect adjacent ecosystems and eliminate resources for people that rely on mangroves for numerous products and services. Furthermore, mangrove destruction can release large quantities of stored carbon and contribute to global warming and other climate change components.

At present, the major threats to mangrove forests include unsustainable felling for wood products, loss of mangrove area for pond aquaculture and over exploitation of plants for traditional usage such as medicine. At present, climate change components such as sea level rise, is likely been a smaller threat than anthropogenic activities, relative sea level

rise is a considerable cause of recent and future reductions in the area and health of mangrove forests. Thus, it is important to assess the effects of sea level rise on mangrove species and ecosystems.

1.2 MANGROVE FORESTS OF MALAYSIA

The diversity of mangrove forests in the ASEAN region, relative to other geographical regions, is considerably higher owing to the tropical climate and sheltered coastlines (Japar, 1994). In this region, a total area of 5,176,695 hectares are covered by the mangrove forests of which, 82.18% is found in Indonesia, 2.05% in Philippines, 0.01% in Singapore, 3.35% in Thailand and 12.40% in Malaysia (Chan et al., 1993). Depending on the inclusion of plant species as exclusive and non-exclusive mangrove species in different countries, a total of 31-34 exclusive species and 40-65 species of non-exclusive species are found in this region (Chan et al., 1993).

In Malaysia, the mangrove forest distribution can be divided between Peninsular Malaysia (West), Sarawak and Sabah (East). The total area of mangrove forests in Peninsular Malaysia is 103, 083 hectares, and 173, 614 and 365,345 hectares in Sarawak and Sabah respectively (Chan et al., 1993). These mangrove forests consist of a total 38 species belonging to 15 different families as exclusive species and 57 species as non-exclusive with 9 species of associated biota. The mangrove forests of the west coast face Malacca Straits while that of east coast face the South China Sea.

In the recent years, 16% of the Malaysian mangrove forests have been lost due to various anthropogenic activities (Chan et al., 1993). However, some states have lost as much as 30-70% of the original areas (Chan et al., 1993). Thus, much attention is devoted to restoration and protection of the mangrove forests.

Numerous studies on various aspects of mangrove forests are constantly carried out in Malaysia. These include, assessment of the plant diversity and community ecology (Ashton and Macintosh, 2002; Rozainah and Aslezaeim, 2010; Saraswathy et al., 2009); evaluation of productivity, plant biomass and nutrient flux in mangrove forests (Alongi et al., 2004; Alongi et al., 1998; Gong and Ong, 1990; Hossain et al., 2008) and rehabilitation and restoration studies in specific sites (Affandi et al., 2010; Hashim et al., 2010; Tamin et al., 2011). Much less attention is given to climate change threats and its impact on mangroves. Hence, with the ongoing climate change, the need to study the responses of mangrove plants to rising sea levels is crucial.

1.3 AIMS AND OBJECTIVES

The predicted climate change is likely to affect different organisms at different scales depending on the ability of the individual species to cope and adapt to the ongoing changes in the atmosphere. Due to the spatial distribution of mangrove plants, where they occupy the interface between the land and the sea, the projected changes in the sea level pose a great threat to these ecosystems. Although, most mangrove species can grow on any tropical shoreline, their permanent establishment is restricted to locations of optimal environmental conditions (Ellison and Stoddart, 1991). The projected climate change for the year 2100 is likely to alter their productivity at local scales by directly affecting photosynthesis and physiology of mangroves as a result of increased temperature and carbon dioxide, and indirectly due to induced stress associated with sea-level rise (Semeniuk, 1994). Hence the broader aim of the current researches is to study the ecological and physiological responses of selected mangrove species under sea level rise.

The experimental evaluations described in the following chapters aims to answer two main questions:

1. Does elevated sea level affect seedling growth and survival of mangrove species?

2. Are there variations in the responses of individual species to elevated sea levels?

The main objectives of the researches are:

1. To study the growth and physiological responses of *A. alba* under simulated sea level rise.
2. To study and compare growth and physiology of *Avicennia marina* var. *acutissima* and *Bruguiera parviflora* under simulated sea level rise.

1.3.1 Specific Research Questions and Objectives

1.3.1.1 Chapter 3

Research question: Does elevated sea level affect growth and survival of *A. alba* seedlings?

Research Objectives:

1. To evaluate germination, establishment and growth of *A. alba* seedlings under simulated sea level rise in the greenhouse under controlled environments.

2. To quantify the growth of *A. alba* seedlings at two intertidal zones in the field under different elevations.

3. To quantify the variations in soil physicochemical characteristics resulting from elevated sea levels.

1.3.1.2 Chapter 4

Research question: Does stimulated sea level rise affect physiological responses of *A. alba*?

Research Objectives:

1. To compare assimilation, stomatal conductance and transpiration of *A. alba* seedlings at ambience under different sea levels.
2. To determine the carbon sink potential of *A. alba* seedlings subjected to sea level treatments.

1.3.1.3 Chapter 5

Research questions:

1. Does stimulated sea level rise affect growth of *Avicennia marina* var. *acutissima* and *Bruguiera parviflora*?
2. Are there variations in the growth responses of *A. marina* var. *acutissima* and *B. parviflora* under simulated sea level rise?

Research Objectives:

1. To quantify growth responses of *A. marina* var. *acutissima* and *B. parviflora* under simulated sea level rise.
2. To compare variations in growth responses of *A. marina* var. *acutissima* and *B. parviflora* under simulated sea level rise.

3. To evaluate if soil physical characteristics depend on sea level treatments and species.

1.3.1.4 Chapter 6

Research questions:

1. Does stimulated sea level rise affect physiological responses of *Avicennia marina* var. *acutissima* and *Bruguiera parviflora*?
2. Are there variations in the physiological responses of *Avicennia marina* var. *acutissima* and *Bruguiera parviflora* under simulated sea level rise?

Research Objectives:

1. To quantify physiological responses of *Avicennia marina* var. *acutissima* and *Bruguiera parviflora* under simulated sea level rise.

2. To compare variations in growth responses of *Avicennia marina* var. *acutissima* and *Bruguiera parviflora* under simulated sea level rise

CHAPTER 2 - LITERATURE REVIEW

2.1 MANGROVE ECOSYSTEMS

Mangrove ecosystems or “mangals” are the forest communities found at the interface between land and sea in the tropics and subtropics (Macnae, 1968). These forests once occupied 75% of the tropical coasts and inlets (Chapman, 1976) and are found in 112 countries and territories (Kathiresan and Bingham, 2001). The latitudinal limits of these ecosystems are 31°22’N in southern Japan, 32°20’N in Bermuda and to the south in Australia 38°45’S, New Zealand 38°59’S and the east coast of South Africa 32°59’S (Spalding et al., 1997). Mangrove ecosystems comprise of taxonomically diverse trees, shrubs, and fern species; and the associated animals, fungi and microbes that are well adapted to straddle between the marine and terrestrial environments (Ball, 1988b; Tomlinson, 1986).

Mangrove ecosystems differ from other ecosystems in various aspects. Their prevalence between the land and sea enable them to occupy various habitats such as tidal flats, beaches, rocky shores, lagoons and sandy island (Semeniuk, 1993). These habitats are subjected to a variety of environmental extremities such as salinity, intense tides, strong winds, high temperatures and various degrees of soil anoxia (Duke et al., 1998a; Field et al., 1998a). Likewise, these forests differ from terrestrial ecosystems due to the lack of a functional understory (Chapman, 1976). The absence of shade tolerant herbaceous, shrubs and vine species in these forests is attributed to a combination of abiotic stresses present at the intertidal environment (Snedakar and Lahmann, 1988)

The plant species diversity in these forests vary across continents (Field et al., 1998a) resulting from variations in precipitation and latitudes (Duke et al., 1998a; Smith and

Duke, 1987). Generally, plant species diversity tends to increase with precipitation (Duke, 1992) and decrease with increasing latitudes (Smith and Duke, 1987). In addition to these, environmental factors such as temperature, salinity, atmospheric carbon dioxide, light, nutrients and tidal inundation determine the diversity and global distribution of mangrove ecosystems (Krauss et al., 2008).

The diversity of dominant higher plants in mangrove ecosystems is relatively low compared to other ecosystems such as rainforests. The term “mangrove” generally refers to the woody halophytes that are solely found in the estuaries and intertidal zones throughout the tropics and subtropics, and are also referred as true mangroves (Tomlinson, 1986). These comprise of 54 species from 20 genera and 16 families (Tomlinson, 1986). It is perceived that these plants first evolved from their terrestrial ancestors possibly after the first angiosperm, around 114 million years ago (Duke, 1992). These plants possess a combination of highly developed morphological, biological, ecological and physiological adaptations to cope with extreme environmental conditions.

2.2 ADAPTATIONS OF MANGROVE PLANTS

As the inhabitants of the interface between land and sea, mangrove plants are exposed to various levels of environmental extremities depending on their location along the intertidal zone. These include strong waves that demand special adaptations to adhere to the substrate while being exposed to intense wave energy (Young and Perkocha, 1994). At high tide, plants may be fully or partially submerged, causing various levels of anoxia resulting from restricted gas exchange between the substrate and the atmosphere (Pezeshki, 2001). Tidal flooding also induces an influx of salt water with high osmotic potentials, emerging the need to regulate salt loads and maintain osmotic balance within

the plants (Ball, 1988b). Furthermore, high temperatures and strong winds at the coastal settings further increase the risks of desiccation as a result of excessive water loss through transpiration. To overcome the constraints resulting from the environment, mangrove plants have evolved several adaptations (Tomlinson, 1986). These adaptations vary among taxa and with the physicochemical characteristics of the habitat (Duke, 1992).

2.2.1 Specialized Root Systems

The root systems of the mangrove plants play important roles in anchoring, gas exchange and nutrient uptake. However, they differ in their position and morphology; with exposed aerial roots to facilitate gas exchange and buttresses that aid firm anchoring in the loose muddy substrates. The aerial roots have enlarged zones of elongation behind the apical meristem and have substantial secondary thickening similar to stems which enables aboveground growth (Kathiresan and Bingham, 2001). The aerial roots consist of lenticels and aerenchyma tissues that allow exchange of gases between the shoot and the roots (Ball, 1988b).

Buttress roots observed in *Xylocarpus* and *Heritiera* are specialized root systems growing on all sides of a mature tree. They do not penetrate deep into the soil and are often in contact with the upper soil layer where all the main nutrients are found (Crook et al., 1997). They form cable like root systems by interweaving with the buttresses of the neighbouring trees resulting in a dense network of support system which prevents the trees from falling over and enabling trees to grow up to heights of 60 meters (Emilio, 1997). The extended buttress roots also enable these species to survive under strong waves by slowing the tidal flow (Young and Perkocha, 1994) and promote deposition of mud and silt (Chapman, 1976). Prop and stilt roots are characteristics of

Rhizophora species. These roots originate from the trunk and lower branches of the trees. They form shallow strands to support the position of the adult tree and are packed with lenticels to facilitate gas exchange (Tomlinson, 1986). More recent findings suggest that these roots generate oxygen by photosynthesis, leading to an increase in oxygen concentrations within these structures during periods of tidal submergence (Kitaya et al., 2002a). The horizontal roots below the soil surface of *Bruguiera*, *Ceriops* and *Xylocarpus* periodically grow vertically upwards forming a loop before continuing their horizontal growth known as root knees (Tomlinson, 1986). At the site of the loop, secondary thickening occurs on the upper side and a structure that resembles a bent knee emerges above the soil surface. Pneumatophores are erect pencil-like roots projecting from the soil surface. They are found in *Avicennia*, *Sonneratia* and *Lumnitzera* resulting from the branching of the horizontal roots under the soil surface (Tomlinson, 1986). They usually appear as single projections but are capable of branching when damaged as observed in the Arabian Gulf where branched pneumatophores are observed following the Gulf War (Boeer, 1993). They also contain chlorophyll in the subsurface layers and conduct photosynthesis (Kitaya et al., 2002b).

2.2.2 Leaf Morphology and Anatomy

The leaf morphology and anatomy of mangrove plants exhibit a variety of xeromorphic characteristics indicating the adaptive responses to survive under physiological drought (Ball, 1988b; Tomlinson, 1986). The leaves generally demonstrate dorsiventral symmetry where upper and lower surfaces of the leaves vary in structure; with the majority of the photosynthetic cells facing upwards to harvest maximum light energy for photosynthesis and the majority of the stomata are saturated on the lower surface to minimize water loss through transpiration (Das and Ghose, 1996).

The cuticles on the leaves of mangrove plants are thick and smooth with wax coatings often with small hairs or epidermal outgrowths that impart a glossy appearance in these plants (Kathiresan and Bingham, 2001). Similarly, these leaves have sunken stomata that retard water loss through transpiration (Kathiresan and Rajendran, 2005).

Mangrove plants bear a number of idioblasts in the leaves that are known as specialized cells found in isolation that differ in their form, size, contents and wall structure (Foster, 1956). The leaves contain branched sclereids that provide mechanical support and provide defence against herbivory (Foster, 1956). The sclereids together with tracheids provide a means of water storage in these plants (Tomlinson, 1986).

Apart from osmotic regulations, leaves of mangrove plants play a significant role in maintaining the internal temperature for efficient photosynthesis (Ball, 1988b). Firstly, mangrove exhibit heliotropism, a phenomenon whereby leaves reorient in response to the irradiance from the sun (Ball et al., 1988). Generally, species that exhibit more conservative water use tend to avoid intense radiation (Ball et al., 1988). Secondly, decreased leaf size enhances boundary layer conductance causing the internal temperature of the leaf similar to ambient air temperature (Ball, 1988b). Thirdly, heat capacity per unit area of the leaf affects the leaf temperature. Among the Rhizophoraceae, heat capacity of the leaves increases with increasing salinity (Camilleri and Ribi, 1983), exposure (Ball et al., 1988) and salinity tolerance of the species (Ball, 1988b).

2.2.3 Reproductive Adaptations

Mangroves; like most terrestrial plants; reproduce by flowering with pollination occurring through wind and insects. However, unlike most terrestrial plants, they do not

produce dormant resting seeds. Mangrove plants exhibit two reproductive adaptations – viviparity and propagule dispersal (Tomlinson, 1986). The precocious and continuous growth of the offspring without the intervention of a resting stage, while being attached to the maternal plant is defined as vivipary in flowering plants (Goebel, 1905). In this sense, mangrove plants produce fully functional "small trees" for which the term propagules is given (Rabinowitz, 1978).

The dispersal process of the propagules is initiated by the gravitational pull that enables the propagules to drop into the water which transports them to great distances. The propagules are buoyant and the presence of a substantial nutrient reserve enables them to float for extended periods without losing their viability (Rabinowitz, 1978). Furthermore, these propagules can survive desiccation and in certain species they can float for over a year before landing on a suitable environment (Rabinowitz, 1978).

2.2.4 Physiology

Mangroves have evolved a number a physiological adaptations to tolerate high salinities, water loss and high light intensities (Ball, 1988b; Kathiresan and Bingham, 2001; Tomlinson, 1986). The specific physiological mechanisms that enable mangroves to survive under extreme environmental conditions vary among individual species where different species exhibit different mechanisms to overcome the stresses. Due to these differential mechanisms, mangrove plants are distributed in a remarkable zonation pattern depending on the abilities of individual species to survive under different environmental gradients (Tomlinson, 1986).

2.2.4.1 Salt Regulation

Mangroves are halophytes that complete their life cycles under saline conditions (Flowers et al., 1986). In the coastal environment, the major constituent of the salinity is sodium chloride (NaCl) and the salinity at a specific site is determined by the local hydrology where periodic tides inputs the salt and the freshwater is supplied from rivers, rainfall, groundwater and runoffs (Ball, 1998). Hence under certain ecological settings, site-specific salinity gradients could vary from freshwater to hyper saline conditions. Due to this, mangrove species exhibit a wide range of growth responses to salinity (Ball and Pidsley, 1988).

Despite the high osmotic potentials of the habitats, mangroves have mechanisms to obtain freshwater at the root surface and tolerate high salt levels (Ball, 1988a). Mangrove plants utilize a combination of salt exclusion, salt excretion and salt accumulation to avoid excessive salt loads (Ball, 1988b). In general, the root cells of the higher plants are highly permeable and take up salt ions readily. Despite this permeability of the root cells, the concentration of NaCl in the xylem sap of mangrove plants are much lower than that of seawater indicating selective exclusion of NaCl at root level (Scholander et al., 1962). Certain genera such as *Rhizophora*, *Bruguiera* and *Ceriops* have ultrafilters in their root systems that selectively reject salts while extracting water from the substrate (Scholander et al., 1962). The concentration of NaCl in the xylem sap of mangrove plants is therefore used as an indicator of salt exclusion in mangrove species.

In other genera such as *Avicennia*, *Acanthus* and *Aegiceras*, the amount of NaCl in the xylem sap is relatively more (Scholander et al., 1962). The species belonging to these genera have mechanisms of salt excretion where 90% of the salts are excreted via the

leaves (Scholander et al., 1962). Salt excretion species have salt glands on the leaves which are visible to the naked eye as minute dimples where salt crystals are formed on the leaf surface as observed in *Avicennia marina* var. *acutissima* (Plate 2.1)

Lumnitzera and *Excoecaria* have succulent leaves where salt is collected in the leaf vacuoles (Kathiresan and Bingham, 2001). The increase in leaf succulence with salinity has been reported in *Laguncularia racemosa* and *Rhizophora mangle* under natural conditions and in *Rhizophora mangle*, *Rhizophora stylosa* and *Avicennia marina* (Camilleri and Ribi, 1983). The amount of salt content in the sap is always higher in the older leaves compared to the younger leaves and leaf succulence increased with leaf age in *Rhizophora mucronata* (Atkinson et al., 1967). Hence, a greater proportion of the salt that is absorbed into the sap is removed via senescing leaves or by storing them in the bark or the wood (Tomlinson, 1986).

Plate 2.1. Salt crystals formed on *Avicennia marina* var. *acutissima*

2.2.4.2 Osmoregulation

Mangroves maintain a positive water balance by keeping the sap water potential at levels lower than those in the root environment (Clough et al., 1982). The osmotic potential required to maintain the water potential within the sap is generated by the accumulation of dissolved solutes in the cell sap (Ball, 1988b). Thus, to facilitate transportation of water from roots to shoots, the water potential of the leaves is maintained at levels lower than that in the roots (Scholander et al., 1962)

The majority of the inorganic ions that function in the osmoregulation of the tissues are accumulated in the cell vacuoles (Ball, 1988b). This separates the salt sensitive enzymes in the cytoplasm from high salinities. The osmotic adjustments in the chloroplasts and other organelles are achieved by the accumulation of compatible solutes that do not interfere with the metabolism (Popp et al., 1985). The selective exclusion of salt at the root level could increase the salinity of the substrate which creates strong osmotic gradients (Passioura et al., 1992). Several mangrove species overcome this problem with the aid of polymeric substances in the sap that limit the flow rate and hence decreasing the transpiration rate (Zimmermann et al., 1994). However, lower transpiration rates and slow water uptake are not common to all mangrove plants (Becker et al., 1997) as seasonal variations also account for variation in transpiration rates (Hirano et al., 1996).

2.2.4.3 Photosynthesis

Mangroves occupy habitats characterized by high irradiation and where they occupy tropical coastlines, they are exposed to continuous high temperatures throughout the year. Hence mangroves need to balance their carbon gain against waterloss and heat stress. As stated earlier, mangrove plants have comparatively low transpiration rates

compared to the terrestrial plants (Lugo and Snedaker, 1974) and the lack of evaporative cooling of the leaf surface causes over heating which is a substantial ecological constraint. Furthermore, mangroves have comparatively lower photosynthetic rates and due to the high irradiation these plants are faced with problems of photoinhibition of the photosynthetic apparatus (Bjorkman et al., 1988).

Mangroves utilize C_3 photosynthetic biochemistry (Ball, 1988b; Tomlinson, 1986) where the amount of water loss through transpiration is equivalent to sustain assimilation rates similar to the photosynthetic capacity, except under extreme conditions when such a balance is not achievable (Ball and Farquhar, 1984). Maximum assimilation rates and stomatal conductance is achieved at leaf temperatures between 25° C to 35° C (Ball et al., 1988). At temperatures above 35° C, both assimilation and stomatal conductance drop abruptly but this decrease may not reduce transpiration rates at an equivalent rate causing water cost of carbon gain to increase with increase in leaf temperatures above optimal (Ball, 1988b).

Mangrove plants exhibit remarkable variations in the photosynthetic activity that enables these plants to balance waterloss and carbon gain. One possible cause for the intra and interspecific variations in the photosynthetic activity of these plants could depend on the genetic variations observed among and within species (Duke et al., 1998b; Lakshmi et al., 1997). This notion is supported by the conclusion that the photosynthetic activity in *Bruguiera* is under direct internal control and not influenced by the changes in salinity or light (Cheeseman et al., 1991).

Alternatively, variations in the photosynthetic activity among species could result from the environmental gradients observed in the habitats. For example, *Avicennia germinans* and *Aegialitis annulata* had reduced carbon losses and increased CO_2 assimilation at

lower salinities (Naidoo and Willert, 1994). Similarly, temperature affects the photosynthetic and transpiration rates (Ball et al., 1988) which influence the overall growth rates in mangroves. Likewise, strong sunlight can inhibit the activities of photosystem II and reduce photosynthesis (Cheeseman et al., 1991). Despite being exposed to high irradiation, photosynthetic rates of mangroves saturate at relatively low light levels. The excess light energy is removed via the xanthophyll cycle (Gilmore and Björkman, 1994) and through the conversion of oxygen to phenolics and peroxidises (Cheeseman et al., 1997).

The existing literature on the photosynthetic variations in mangrove species, therefore, indicates that mangrove plants may utilize a number of photosynthetic adaptations to maximize productivity at different environmental gradients. These adaptations vary from species to species; and these adaptations may result from genetic variations or may be influenced by the environmental gradients.

2.3 CLIMATE THREATS TO MANGROVE ECOSYSTEMS

The current climate change poses great threats to a number of organisms at various scales. A number of ecosystems and ecological processes have already been affected by the current anthropogenic climate change. Of these, mangrove ecosystems are recognized as one of the most threatened ecosystems, firstly, because large proportions of the mangrove forests are being lost at rates impossible to retrieve (Valiela et al., 2001) and secondly any minute variations in the global environmental conditions may cause adverse changes in the habitats of these unique ecosystems causing a number of ecological imbalances within mangrove ecosystem (Gilman et al., 2008).

2.3.1 Sea Level Rise

One of the observed outcomes of the current climate change is the global sea level rise. It is estimated that the global rise in sea level over the last century is between 12-22 cm (Solomon et al., 2007), and the range that is projected by the end of 21st century is 0.4-1.0 m (Solomon et al., 2009). This increase in sea level, relative to a fixed Earth Coordinate System, is primarily a result of thermal expansion of sea water and the transfer of ice from glaciers, ice sheets and ice caps into the ocean (Church et al., 2001).

The rising sea level is the most significant climate change component that is likely to affect mangrove ecosystems (Ellison, 1993; Ellison and Stoddart, 1991; Gilman et al., 2008). The majority of the mangrove ecosystems lie on sedimentary shorelines of gentle slope, between mean sea level (MSL) and the level of mean high water spring tides (Ellison, 2000). The mangrove forests are highly vulnerable to rise in sea level because of their existence in the upper half of the tidal range (Ellison, 2000). Since the majority of the mangrove forests are found in the Pacific islands where the tidal range is less than one meter, a sea level rise of 0.3 m is likely to cause a substantial effect on the mangrove forests. The mangrove forests of the low islands where there is no external supply of sediment are most susceptible to rising sea levels (Ellison, 1993). Similarly, sea level rise coupled with erosion of mangrove substrate in the low islands could already affect mangroves of these islands (Ellison, 2000).

The major ecological constraint of the sea level rise to mangroves is prolonged tidal inundation. Flooding in mangrove forests can range from tidal flooding where mangroves are subjected to daily semi diurnal tides to near permanent flooding at the lower intertidal zones (Watson, 1928). Tidal flooding restricts gaseous exchange between the substrate and the atmosphere causing soil anoxia (Tomlinson, 1986).

Mangroves respond to flooding by changing the internal biochemical processes and by developing lenticels on the basal stems or root structures that aid in gaseous exchange (Tomlinson, 1986). However, given the distinct zonation patterns observed in the intertidal mangrove forests, it is evident that individual species respond to tidal inundation differently, and have been the focus of a few recent experimental studies (He et al., 2007; Kitaya et al., 2002a; Krauss et al., 2006b; Ye et al., 2010). The species that prevail in the lower intertidal zone is more likely to be tolerant to sea level rise whereas those species that are found on the landward margin will be severely affected under prolonged tidal inundation.

The site specific sea level rise relative to the elevation of the mangrove sediment surface is likely to retreat mangrove forests landwards to maintain their preferred hydroperiod (Gilman et al., 2008). The landward margin of the mangrove forests may migrate into higher elevations if the environmental conditions such as hydrology and sediment composition, competition with non mangrove plants and availability of waterborne seedlings prevail in the landward areas (Krauss et al., 2008). Likewise, salt water intrusions into the freshwater river floodplains cause death of freshwater wetland communities and upstream invasion of mangroves (Ross et al., 1994; Woodroffe, 1995).

The seaward mangrove margin migrates landward as a result of tree dieback resulting from stresses associated with sea level rise such as prolonged tidal inundation and exposure to salinity (Ellison and Farnsworth, 1993; Naidoo, 1983). However, the extent to which these forests retreat depends on the ability of individual species to establish on the newly available habitat at a rate that keep pace with the rate of relative sea level rise (Duke et al., 1998a; Field, 1995; Lovelock and Ellison, 2007). Similarly, the overall persistence of mangrove forests as global sea level rise is dependent on the shoreline

geomorphology, sedimentation and the actual rate of sea level rise for a particular habitat (Gilman et al., 2008; Semeniuk, 1994).

2.3.2 Atmospheric Carbon dioxide (CO_2)

The concentration of the atmospheric CO_2 has increased from a pre-industrial value of 280 ppm to over 380 ppm today (Solomon et al., 2007). This increase in the atmospheric CO_2 concentration is largely a result of burning fossil fuels together with land-use changes and other industrial processes (IPCC, 2001). CO_2 directly affects plant growth and development where elevated CO_2 is likely to increase net primary productivity in ecosystems resulting from enhanced photosynthesis (Bazzaz, 1990; Urban, 2003). Similarly, CO_2 affects stomatal regulation of water loss where many species reduce stomatal aperture and increase photosynthetic water use efficiency (PWUE: carbon gain per unit of water lost) under elevated CO_2 (Bazzaz, 1990; Urban, 2003).

In mangroves, the potential responses to CO_2 is dependant upon the activity of carbon sinks; the growing tissues; and carbon reserves such as the cotyledons (Krauss et al., 2008). Similarly, CO_2 enrichment is likely to alter susceptibility to photoinhibition and herbivores resulting from the variations in tissue quality such as increased non-structural carbohydrates and decreased nitrogen (Poorter et al., 1997). However, the extent to which plants benefit from increased CO_2 is limited by other factors such as nutrient availability. In general, plants exhibit a greater absolute response to elevated CO_2 under increased nutrient availability. Similarly, it is predicted that CO_2 enrichment will enhance stress tolerance and competitive ability in mangrove plants.

There are only a few studies that quantify the predicted effects of elevated CO_2 on mangrove plants. For example Farnsworth et al. (1996) studied the growth and

physiological responses of *R. mangle* seedlings that were subjected to 350 ppm and 700 ppm for over a year. Elevated CO_2 increased biomass, total stem length, branching activity and total leaf area. Similarly, higher relative growth rates and net assimilation rates were observed under increased CO_2. The initial photosynthetic rates varied among the CO_2 treatments where CO_2 enrichment increased the maximum photosynthetic rates. However, this difference was not significant towards the end of the study. Furthermore, increased CO_2 stimulated development of aerial prop roots and reproductive buds.

In another study, seedlings of *R. apiculata* and *R. stylosa* were subjected to CO_2 concentrations of 340 ppm and 700 ppm together with salinities of 25 and 75%; and humidity levels of 43 and 85% (Ball et al., 1997). Enhanced growth was observed through increased net assimilation at low salinities especially in *R. apiculata* which is less salt tolerant than *R. stylosa*. However, CO_2 enrichment had little effect on mangrove growth rates in higher salinities. Thus, the greatest effects may be observed under low salinity conditions.

Effects of CO_2 on *A. germinans* was studied at CO_2 concentrations of 365ppm and 720 ppm and in the presence and absence of a *Spartina alterniflora*; a C_4 grass; and under two nitrogen levels (McKee and Rooth, 2008). *A. germinans* had enhanced growth and biomass when grown alone especially at higher nitrogen levels. However, seedling growth was substantially reduced when grown with *S. alterniflora* and CO_2 enrichment and nitrogen availability did not promote growth of *A. germinans*. Hence, competition from other species may hinder mangrove seedling response to CO_2.

The available literature on mangrove plant responses to elevated CO_2, therefore, indicate increased productivity through enhanced photosynthesis and water use efficiency when subjected to CO_2 enrichment under optimal conditions. However, CO_2

responses depend on other growth limiting factors such as salinity and nutrient availability. Similarly, competition from other species may limit mangrove seedling responses to CO_2 and it is not indicative if elevated CO_2 is likely to increase stress tolerance and competitive abilities of mangrove species. Likewise, current literature is based on studies that employ individual species and not all species are likely to respond in a similar manner given the taxonomic diversity and inherent characteristics of the mangroves. Furthermore, these studies focus on mangrove plants grown in isolation, thus little is known on the effects of CO_2 on the mangrove species in the presence of a combination of environmental extremities and when in competition with coexisting plants.

2.3.3 Temperature

The global average surface temperature has increased steadily at a rate of 0.13° C per decade for the last 50 years; a value twice that for the last 100 years (Solomon et al., 2007). This rise in global temperature is most likely caused by the emission of greenhouse gases into the atmosphere that absorb thermal radiation from the planetary surface causing the temperature of the earth to increase (Solomon et al., 2007). By the end of 21st century, the global surface temperature is likely to increase by 1.1-6.4° C (Solomon et al., 2009).

The increase in surface temperature is likely to change species composition of the mangrove forests (Field, 1995). Mangrove forests border a latitudinal limit at the 16° C isotherm for air temperature of the coldest month, at the margins of the incidence of ground frost, and where water temperature do not exceed 24° C (Ellison, 2000). With global warming, the diversity of mangrove plants is likely to increase in the higher latitudinal margins (Ellison, 2000). At present the predominant species at these limits is

Avicennia, however, with global warming, other mangrove species are likely to expand their range into these mangrove margins (Ellison, 2000). Similarly, the rise in surface temperature will expand mangrove ranges to higher latitudes where range is limited by temperature but not by other environmental conditions including availability of propagules and suitable physiographic conditions (Ellison, 2000). Furthermore, the current increase in global temperature is likely to increase the productivity of mangroves through increased growth and litter production at all localities (Ellison, 2000).

The assimilation rate of mangrove plants is optimal at leaf temperatures between 28-32° C, and at temperatures exceeding 35° C the photosynthetic activity is lost (Clough et al., 1982). For example, root transpiration rate of *R. mangle, A. germinans* and *L. racemosa* seedlings increased linearly with temperatures between 20-45° C (McKee, 1996). The data indicated a substantial change in root metabolism near 30° C for all three species indicating that minor changes in soil temperature could have a large effect on relative growth of mangrove seedlings. In another study, at temperatures beyond 37° C, root development of *A. germinans* seedlings from coastal Texas was inhibited (McMillan and Sherrod, 1986).

An indirect effect of global warming on ecosystems is the change in phenological patterns of a number of organisms (Menzel et al., 2006; Parmesan and Yohe, 2003). Phenology is the timing of periodic events in an organism and these events are highly dependent on seasonal and annual variations in climate. From 1971 to 2000 a 30% significant advancement in the leafing, flowering and fruiting in 78% of 542 plants have been reported (Menzel et al., 2006). This trend is consistent with the current global warming. Such untimely variations in the phenological events among species that coexist under mutualism are likely to raise a number of ecological concerns especially when pollination depends on animals such as small insects, moths, bats and birds

(Kathiresan and Bingham, 2001). Therefore, it is worth considering the number of mangrove species that solely rely on these animals for pollination. Hence, phenological variations may hinder pollination, flowering and reproduction in mangroves. However, the extent to which these events vary is still poorly understood.

The predicted increase in the global surface temperature will directly influence the photosynthesis in plants and is likely to increase productivity and diversity in the mangrove forests. However, increase in global temperatures may affect ecological interaction such as the plant-pollinator interactions of organisms in a negative manner. The difference in the timing of phenological events such as the emergence of the pollinators and flowering in plants could affect propagule availability and therefore the population dynamics of mangrove forests. The balance between the benefits and costs of direct and indirect effects of increased temperature is poorly understood and is highly debated among scientists.

2.3.4 Precipitation

The mangroves forests of the world prevail in the warm humid regions, such as the equatorial and tropical summer rainfall regions (Ellison, 2000). In these regions, mangrove forests are taller and more diverse compared to drier coastlines where mangrove forests are low, scattered or sporadic (Duke et al., 1998a). The predominant abiotic factor that determines the diversity of these forests is the rainfall pattern, where diversity increases with increased rainfall (Ellison, 2000). This observed trend is related to salinity stress where increased precipitation leaches the soil and increases availability of freshwater (Ellison, 2000). Furthermore, heavy rains let river discharge and groundwater overflow which provide nutrients (Ellison, 2000; McKee, 1993). In

contrast, in arid intertidal zones, the salinity is increased due to evaporation resulting in poor vegetation structures in mangroves (Ellison, 2000).

The global rainfall pattern coupled with current climate change is predicted to increase by 25% by 2050 (Gilman et al., 2008). However, regional distribution of rainfall is likely to vary (Houghton et al., 2001) where increased rainfall is more likely in high latitudes and decreased rainfall in most subtropical regions (Solomon et al., 2009). Consistent with these predictions, increased rainfall over the equatorial Pacific has been observed over the last few decades (Houghton et al., 2001).

The decreased rainfall in the subtropics is expected to affect mangrove distribution in these regions (Ellison, 2000; Field, 1995). Decreased rainfall and increased evaporation will increase salinity resulting in decreased productivity, growth and seedling survival, diversity and reduced mangrove area (Duke et al., 1998a; Field, 1995). Hence, reduced precipitation could facilitate mangrove encroachment into salt marshes and freshwater wetlands (Rogers et al., 2005; Saintilan and Wilton, 2001).

Mangrove forests in regions where precipitation is expected to increase will have increased productivity and biodiversity (Duke et al., 1998a). Hence, mangroves may expand into the open areas of the landward fringe within tidal wetland zone (Field, 1995). Furthermore, increased freshwater coupled with decreased salinity is likely to decrease exposure to sulphate causing increased peat production in mangrove forests (Snedaker, 1995). Hence, variation in precipitation patterns is likely to affect global distribution of mangroves. Nonetheless, further research is needed to evaluate these hypotheses and to determine the effects of rainfall variability on mangroves.

CHAPTER 3 - EFFECTS OF SEA LEVEL RISE ON THE ESTABLISHMENT AND EARLY GROWTH OF *AVICENNIA ALBA*

3.1 INTRODUCTION

Numerous greenhouse studies under simulated flooding provide ample data on seedling growth and survival of mangrove species (Ellison and Farnsworth, 1997; Wang et al., 2007; Ye et al., 2004). Field studies on the effects of tidal inundation on *Rhizophora apiculata* and *Bruguiera gymnorrhiza* (Sukardjo, 1987), *Rhizophora mangle*, *Avicennia germinans* and *Laguncularia racemosa* (McKee, 1995) have been reported. Lower intertidal elevations decreased survival of *Avicennia marina*, *B. gymnorrhiza*, *Ceriops tagal* and *Rhizophora stylosa* (Smith, 1987). However, growth of *Avicennia germinans* seedlings were negatively affected either by increased or decreased flooding from the mean water level while growth of *Rhizophora mangle* was better at lower intertidal positions (Ellison and Farnsworth, 1993). These observations, nevertheless, could be affected by several confounding factors in the environment (Komiyama et al., 1996). Hence simultaneous quantification of growth responses under sea level rise in the natural environments and in the greenhouse under controlled environmental conditions is vital to comprehend ecological responses of species to sea level rise.

In Malaysia, 564,606 ha of the coastal area are covered by the mangrove forests of which 16% is in Peninsular Malaysia (Shamsuddin and Nasir, 2005). These include the mangrove forest reserves surrounding the Klang isles in the Strait of Malacca which sustain a high diversity of flora and fauna (Sasekumar and Chong, 2006). The largest of these islands is the Carey Island which is separated from the mainland by the Langat River. The mangrove forests in this islet comprise of 16 species from 5 different

families; of which *Avicennia alba* is the most dominant species (Saraswathy et al., 2009). Despite its abundance little is known on the effects of sea level rise on growth of *A. alba* seedlings. Hence, the aim of the current research is to study the effects of sea levels on the establishment and growth of *A. alba* seedlings. The specific objectives of the study are to evaluate germination, establishment and growth of *A. alba* seedlings under simulated sea level rise in the greenhouse and to quantify the growth of *A. alba* seedlings at two intertidal zones with different sea levels in the field. Determining the effects of sea levels on survival and growth of mangrove seedlings is important for successful plantation of seedlings for rehabilitation and afforestation in the context of a changing environment.

3.2 MATERIALS AND METHODS

3.2.1 Greenhouse Experiment

3.2.1.1 Experimental Design

Three plastic tanks measuring 182 cm long x 122 cm wide x 60 cm deep were used to set up artificial tidal tanks simulating daily semidiurnal tides with two “high” and “low” tide periods, in a greenhouse at the botanical garden of the Institute of Biological Sciences, University of Malaya. Artificial seawater with salinity of 30 ‰ was prepared by dissolving commercial sea salt. Salinity was maintained throughout the study period, either by adding salt or water whenever required. Seawater was pumped into the tanks at “high tide” during 01:00 to 9:00 and 13:00 to 21:00 and ebbed at “low tide” during 09:00 to 13:00 and 21:00 to 01:00. Hence, the seedlings were subjected to 16 and 8 hours of daily high and low tides respectively.

Two treatments were specified; deep and shallow. In order to minimize the "block" effect; each tank accommodated both treatments (Figure 3.1). The plants in the shallow treatment were placed on 30 cm high benches, inside the tank. At high tide, the water level of deep and shallow treatment was 30 cm and 0 cm (up to the rim of the pots) above the soil surface, respectively. At low tide, the water level of deep treatment was up to the rim of the pots and in shallow treatment pots were completely drained. Sea water in the tanks was replaced monthly with newly prepared sea water.

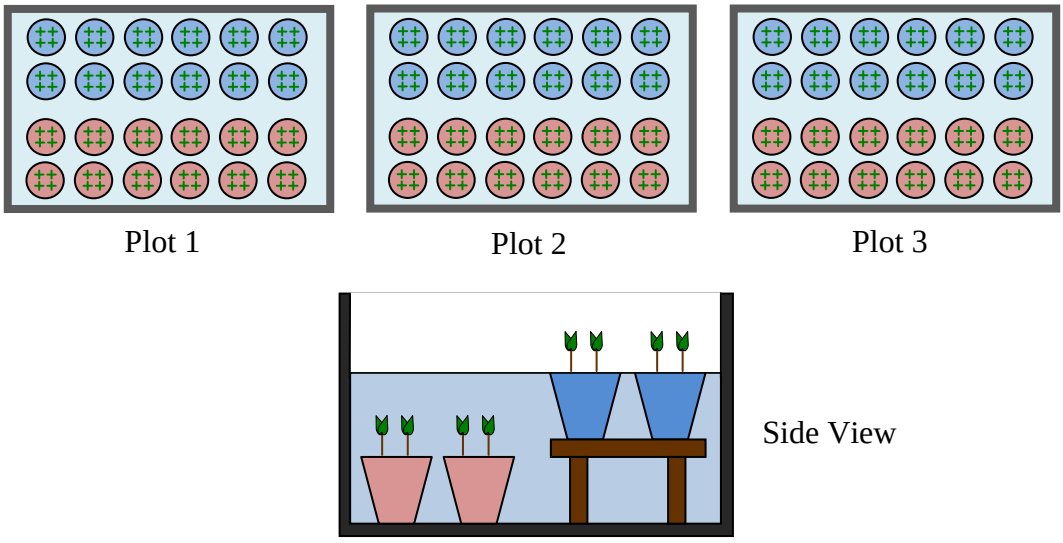


Figure 3.1. Experimental design: Randomized Block Design (RBD)

3.2.1.2 Root development and Establishment of propagules

The effects of sea levels on root development and establishment were studied using mature propagules of *A. alba*. Healthy mature propagules of length 3 to 4 cm (Plate 3.1) were handpicked from the mother trees at the study site in Carey Island, where the field

study was conducted. The propagules were planted upright with half the propagule inserted into the soil (Plate 3.2), at a density of four fruits per pot (n = 288). Daily observations were made to monitor root development and establishment rates. The rupturing of pericarp and visibility of dark green viviparous seed with erupted cotyledons were considered as root development (Plate 3.3). Establishment was observed with the unfurling of the first pair of leaves (Plate 3.4).

3.1. Mature propagules of *A. alba*

Plate 3.2. *A. alba* propagules planted with half the propagule inserted into the soil

Plate 3.3. Root development - dark green viviparous seeds with erupted cotyledons. (A) Root development in *A. alba* in the greenhouse. (B) Single propagule with erupted cotyledons showing the rootlets

Plate 3.4. Establishment – unfurling of the first pair of leaves

3.2.1.3 Seedling growth and survival

Plastic pots measuring 20 cm in diameter and 18 cm in height were filled up to the rim with soil collected from the study site. Soil used in this experiment was collected three days prior to seedling collection. Established seedlings, with 2 – 4 fully expanded leaves and 6 – 9 cm in height were carefully harvested from the study site (Plate 3.5). Four

seedlings were planted in each pot with their first stem nodes just above the soil surface. Initial height, diameter at the first stem node and number of leaves were recorded. The plants were acclimatized for five days by flooding the pots up to the rim.

At the onset of the experiment, the pots were randomly assigned to each of the two treatments. In each tank, 24 pots were placed (12 pots per treatment). Diameter at first stem node was recorded to the nearest 0.01mm using a digital calliper. Total increase in leaf number was defined as, double the number of stem nodes because *A. alba* produce opposite leaves with two leaves per node. Leaf fall was determined from the difference between the total number of leaves and the leaves that remained on the seedling. Survival of the seedlings in both treatments was recorded.

Plate 3.5. *A. alba* seedlings with 2-4 leaves and 6-9 cm in height

3.2.1.4 Biomass allocations and RGR

In order to determine the initial biomass of the seedlings, 36 seedlings were randomly selected at the onset of the experiment. The seedlings were washed and separated into roots and shoots (stem and leaves). Separated components were oven dried at 70 °C for one week to determine the initial biomass. The cotyledons were removed and excluded in the biomass measurements. Subsequent measurements were recorded every three months. Six plants from each treatment in each tank were randomly harvested after three and six months, to determine biomass allocation and relative growth rates (RGR) of the seedlings subjected to the tidal treatments. The RGR was calculated as:

$$RGR = (\overline{\ln W_2} - \overline{\ln W_1}) / t$$

where $\overline{\ln W_1}$ and $\overline{\ln W_2}$ are the means of the natural log-transformed biomasses at the beginning and end of the experiment, respectively; and t is the duration (Hunt, 1982).

3.2.1.5 Soil physical parameters

Soil physical parameters; soil redox potential (Eh) and pH was recorded at the end of the study period to determine any differences in soil physical parameters resulting from different sea levels. For each treatment, 18 pots with a growing seedling were randomly chosen to collect soil pH and Eh. Soil Eh was measured by inserting a multi-parameter probe (Professional Plus, YSI incorporated, Ohio, USA) at a depth of 5 cm into the soil (Ye et al., 2004). Soil pH was recorded using a pH meter (IQ 170, IQ Scientific Instruments, California, USA)

3.2.2 Field experiment

3.2.2.1 Study site

Field study was conducted in an open seaward mangrove fringe (2°49’56.5” N, 101°19’25.6” E) in Carey Island, Malaysia (Plate 3.6). The mangrove fringe is separated from the mainland vegetations which comprises mainly of palm oil plantations by the coastal protection bund. The predominant species found at the site were *A. alba* and *Sonneratia alba*; two colonizing species often found on newly formed mudflats in South East Asia (Panapitukkul et al., 1998).

Plate 3.6. Map of study site: the predominant species were *A. alba* and *S. alba*

The effects of the sea levels on *A. alba* seedlings were studied at two intertidal zones; lower intertidal (LI) and upper intertidal (UI) zones. The two zones were 40 m apart and the sea level in the LI zone during high tide was approximately 30 cm higher than the UI zone. In each intertidal zone, three replicate plots that were 15 m apart were established. In each plot 36 seedlings of *A. alba* (n = 216) from the same cohort, with 2 – 4 fully expanded leaves and 6 - 9 cm tall (Plate 3.7) were tagged and monitored for six months.

3.2.2.2 Seedling growth and survival

The initial growth measures; stem height, diameter at first stem node and number of leaves of the tagged seedlings were recorded in both zones at the onset of the field trials. Increments in growth measures were recorded monthly for six months using the same methods as in the greenhouse experiment.

Plate 3.7. *A. alba* seedling with 4 fully expanded leaves at the study site

3.2.2.3 Biomass allocations and RGR

Six seedlings from each plot were randomly harvested at the beginning of the field trials to determine the initial biomasses of the seedlings in both zones. Biomass allocations and RGR of the seedlings in both zones were recorded every three months by harvesting six seedlings randomly from each plot (n = 36). Similar protocols as in the greenhouse experiment were followed.

3.2.2.4 Soil physical parameters

Soil samples were collected from each plot in both intertidal zones at the beginning of the experiment. Particle Size Distribution (PSD) analysis was performed using a Zetasizer Nano ZS (Malvern, Japan) by dissolving 2 g of soil in deionized water. Soil pH at a depth of 5 cm was recorded at six random points in each plot using a pH meter (IQ 170, IQ Scientific Instruments, California, USA).

3.2.3 Data Analyses

Independent sample t-test was performed to determine any significant differences in growth responses of *A. alba* seedlings resulting from differences in sea levels. Correlation analyses between soil physical parameters and growth responses were performed wherever a significant difference in soil physical parameter was observed. All analyses were completed using the STATISTICA 9.0 (StatSoft Inc., USA).

3.3 RESULTS

3.3.1 Greenhouse Experiment

3.3.1.1 Root development and establishment of propagules

All the propagules of *A. alba* had ruptured pericarp and visible viviparous seeds within 13 days after subjecting to the treatments. Rate of root development was much faster in deep treatment and 100% root development was observed on the 9^{th} day, whereas in shallow treatment 100% root development was observed on the 13^{th} day (Figure 3.2) In contrast, rate of establishment was similar in both treatments (Figure 3.3); however, the number of propagules that established in the deep treatment was significantly less ($t_{2(286)}$

= 3.68, p <0.001) and none of the propagules established after 22nd day. In shallow treatment establishment was observed till 28th day.

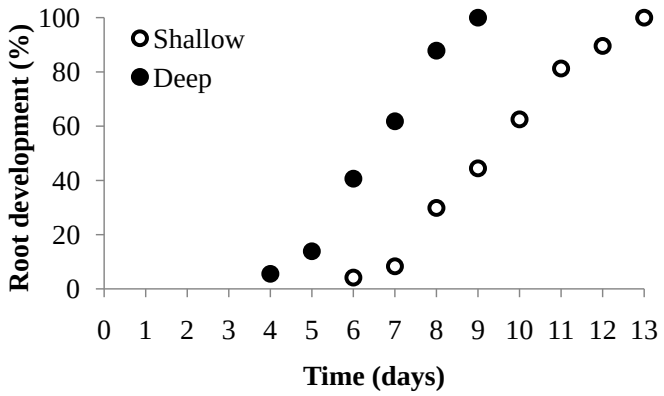


Figure 3.2. Root development of *A. alba* seedlings in two treatments (n = 288)

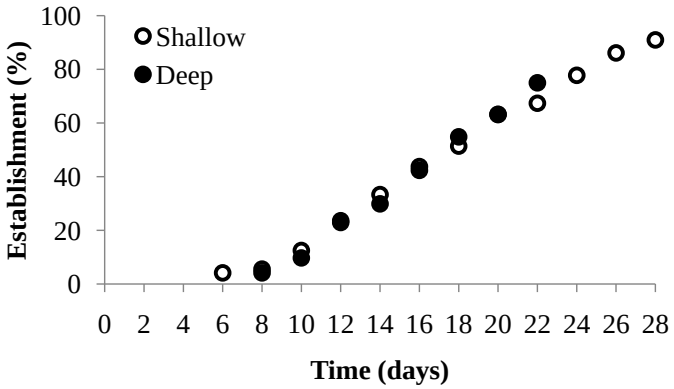


Figure 3.3. Establishment of *A. alba* seedlings in two treatments (n = 288)

3.3.1.2 Seedling growth and survival

The initial growth measures of the seedlings were similar (Table 3.1). High sea level in the deep treatment stimulated height increment of *A. alba* up to third month but no significant differences were observed thereafter (Table 3.1). Stem diameter of the *A.*

alba seedlings in the deep treatment was significantly lower during the last two months (Table 3.1). Leaf formation in both treatments did not show any significant difference up to the fifth month. However, in the deep treatment a significant decrease in leaf formation was observed during the last two months (Table 3.1). Similarly, leaf fall increased in the deep treatment during the last two months (Table 3.1). Poor survival of *A. alba* seedlings was observed in both treatments in the greenhouse (Figure 3.4). At the end of the six months, fewer seedlings survived in the deep treatment compared to the shallow treatment, however this difference was not significant ($t_{2(286)} = 1.78$, $p = 0.076$).

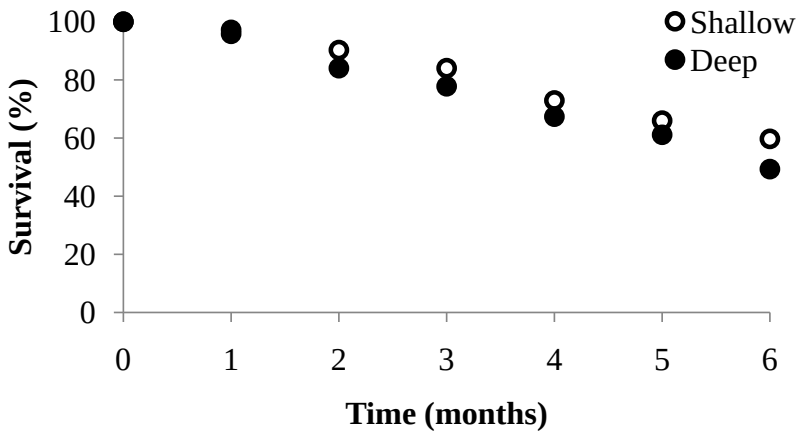


Figure 3.4. Survival of *A. alba* seedlings in two treatments (n = 288)

Table 3.1. Growth responses of *A. alba* seedlings subjected to two tidal treatments in the greenhouse

Growth Parameter	Treatment	Time (months)						
		0	1	2	3	4	5	6
Height (cm)	**Shallow**	7.1 ± 0.8 [a]	7.8 ± 0.9 [a]	8.7 ± 0.9 [a]	9.7 ± 1.0 [a]	10.9 ± 1.0 [a]	11.9 ± 1.1 [a]	13.0 ± 1.3 [a]
	Deep	7.2 ± 0.8 [a]	8.2 ± 0.8 [b]	9.4 ± 0.9 [b]	10.5 ± 1.0 [b]	11.0 ± 1.0 [a]	12.0 ± 1.0 [a]	12.9 ± 1.0 [a]
Stem diameter (mm)	**Shallow**	4.08 ± 0.66 [a]	4.36 ± 0.68 [a]	4.65 ± 0.63 [a]	4.94 ± 0.62 [a]	5.24 ± 0.66 [a]	5.66 ± 0.66 [a]	5.96 ± 0.63 [a]
	Deep	4.00 ± 0.58 [a]	4.26 ± 0.61 [a]	4.50 ± 0.58 [a]	4.83 ± 0.60 [a]	5.19 ± 0.59 [a]	5.39 ± 0.59 [b]	5.50 ± 0.66 [b]
Number of new leaves	**Shallow**	3.5 ± 0.9 [a]	4.9 ± 1.3 [a]	6.6 ± 1.4 [a]	8.4 ± 1.6 [a]	10.3 ± 1.8 [a]	12.1 ± 1.9 [a]	13.8 ± 2.0 [a]
	Deep	3.4 ± 0.9 [a]	4.8 ± 1.1 [a]	6.7 ± 1.1 [a]	8.1 ± 1.4 [a]	10.1 ± 1.6 [a]	11.9 ± 1.5 [a]	12.9 ± 1.7 [b]
Leaf fall	**Shallow**	1.0 ± 0.7 [a]	2.2 ± 1.2 [a]	3.3 ± 1.5 [a]	4.2 ± 1.6 [a]	5.2 ± 1.7 [a]	6.1 ± 1.8 [a]	7.0 ± 1.8 [a]
	Deep	0.9 ± 0.7 [a]	2.0 ± 1.1 [a]	3.0 ± 1.1 [a]	4.1 ± 1.2 [a]	5.4 ± 1.3 [a]	6.8 ± 1.3 [b]	8.3 ± 1.4 [b]

Mean and standard deviation, means in the same column with different letters in the same column are significantly different at α 0.05

3.3.2 Biomass allocations and RGR

The initial biomasses of the seedlings in both treatments were similar (Table 3.2). Similarly, biomass allocations of *A. alba* were not affected by the sea level treatments during the first three months (Table 3.2). However, total, shoot and root biomasses of *A. alba* seedlings was significantly lower whilst a significant increase in root to shoot (R/S) ratio was observed during the last three months in the deep treatment (Table 3.2).

A similar trend was observed in RGR (Figure 3.5), where no significant differences were observed between the treatments during the first three months ($t_{2(34)}$ = 0.43, p = 0.671). However, at the end of six months, RGR of *A. alba* in the deep treatment was significantly lower ($t_{2(34)}$ = 2.65, p = 0.012).

Table 3.2. Biomass allocations of *A. alba* seedlings subjected to two tidal treatments in the greenhouse (n = 36)

Biomass	Treatment	Time (months)		
		0	3	6
Total (g)	Shallow	0.53 ± 0.10 [a]	0.93 ± 0.14 [a]	1.75 ± 0.17 [a]
	Deep	0.49 ± 0.08 [a]	0.91 ± 0.17 [a]	1.42 ± 0.15 [b]
Root (g)	Shallow	0.22 ± 0.05 [a]	0.39 ± 0.08 [a]	0.70 ± 0.06 [a]
	Deep	0.20 ± 0.04 [a]	0.38 ± 0.08 [a]	0.62 ± 0.07 [b]
Shoot (g)	Shallow	0.31 ± 0.06 [a]	0.54 ± 0.09 [a]	1.04 ± 0.13 [a]
	Deep	0.29 ± 0.06 [a]	0.53 ± 0.10 [a]	0.80 ± 0.09 [b]
R/S	Shallow	0.70 ± 0.12 [a]	0.74 ± 0.14 [a]	0.68 ± 0.07 [a]
	Deep	0.69 ± 0.10 [a]	0.71 ± 0.09 [a]	0.79 ± 0.08 [b]

Mean and standard deviation, means in the same column with different letters in the same column are significantly different at α 0.05

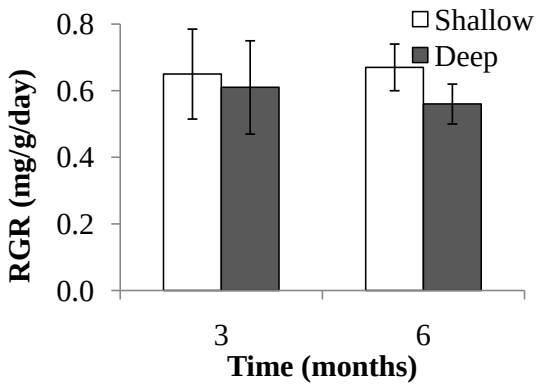


Figure 3.5. Relative growth rates of *A. alba* seedlings in two treatments (n = 36)

3.3.2.1 Soil physical parameters

Sea level treatments in the greenhouse affected soil physical parameters in the pots. The mean Eh of the shallow and the deep treatment was -91.61 and -129.94 respectively ($t_{2(34)}$ = 9.78, p <0.001). Similarly, soil pH in shallow (6.29) and deep (5.26) treatment was significantly different ($t_{2(34)}$ = 7.89, p <0.001). No significant correlation was observed between Eh and RGR (r = 0.21, p = 0.227, n = 36). However a significant negative correlation was observed between Eh and R/S ratio (r = -0.41, p = 0.013, n = 36). Similarly, soil pH had a significant negative effect on R/S (r = -0.52, p = 0.001, n = 36). In contrast, soil pH had a significant positive effect on RGR (r = 0.43, p = 0.009, n = 36).

3.3.3 Field experiment

3.3.3.1 Seedling growth and survival

The results of the field study showed a similar trend as those in the greenhouse experiment. High sea level in the LI zone stimulated height increment of *A. alba* up to fourth month but

no significant differences were observed during the last two months (Table 3.3). Tidal elevation did not affect stem diameter of the seedlings in both zones up to the fourth month, but decreased significantly in the LI zone during the last two months (Table 3.3). Leaf production in *A. alba* was not affected by the tidal elevations during the first three months, but a significant decrease was observed in the LI zone during the last three months (Table 3.3). Likewise, a significant increase in leaf fall was observed in the LI zone during the last four months (Table 3.3). At the end of six months, survival of *A. alba* seedlings was significantly reduced in the LI zone (Figure 3.6) ($t_{2(216)} = 2.65$, $p = 0.009$).

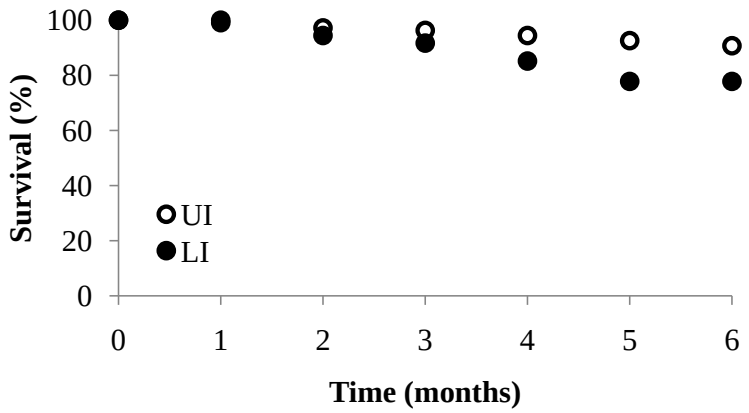


Figure 3.6. Survival of *A. alba* seedlings in two intertidal zones (n = 216)

Table 3.3. Growth responses of *A. alba* seedlings subjected to two intertidal zones in the field

Growth Parameter	Intertidal zone	Time (months)						
		0	1	2	3	4	5	6
Height (cm)	**UI**	6.9 ± 0.7 [a]	7.7 ± 0.8 [a]	8.4 ± 0.9 [a]	9.4 ± 1.0 [a]	10.3 ± 1.0 [a]	11.4 ± 1.0 [a]	12.3 ± 1.1 [a]
	LI	7.0 ± 0.8 [a]	8.0 ± 0.8 [b]	9.1 ± 0.8 [b]	10.3 ± 0.9 [b]	11.0 ±1.0 [b]	11.7 ± 1.1 [a]	12.2 ± 1.1 [a]
Stem diameter (mm)	**UI**	4.04 ± 0.63 [a]	4.29 ± 0.62 [a]	4.63 ± 0.63 [a]	4.90 ± 0.64 [a]	5.20 ± 0.66 [a]	5.61 ± 0.68 [a]	5.91 ± 0.63 [a]
	LI	3.89 ± 0.75 [a]	4.16 ± 0.75 [a]	4.46 ± 0.76 [a]	4.75 ± 0.79 [a]	5.01 ± 0.81 [a]	5.28 ± 0.86 [b]	5.60 ± 0.86 [b]
Number of new leaves	**UI**	3.4 ± 0.9 [a]	4.9 ± 1.0 [a]	6.5 ± 1.1 [a]	8.2 ± 1.3 [a]	10.1 ± 1.5 [a]	11.9 ± 1.5 [a]	13.8 ± 1.5 [a]
	LI	3.6 ± 0.8 [a]	5.0 ± 1.0 [a]	6.7 ± 1.1 [a]	8.3 ± 1.3 [a]	9.5 ± 1.4 [b]	11.0 ± 1.4 [b]	12.0 ± 1.4 [b]
Leaf fall	**UI**	1.0 ± 0.7 [a]	2.0 ± 0.9 [a]	3.0 ± 1.1 [a]	3.7 ± 1.1 [a]	4.2 ± 1.2 [a]	4.9 ± 1.3 [a]	5.6 ± 1.4 [a]
	LI	0.9 ± 0.6 [a]	1.8 ± 0.8 [a]	3.2 ± 1.1 [a]	4.2 ± 1.2 [b]	5.2 ± 1.4 [b]	6.2 ± 1.5 [b]	7.1 ± 1.5 [b]

Mean and standard deviation, means in the same column with different letters in the same column are significantly different at α 0.05

3.3.3.2 Biomass allocations and RGR

The initial biomasses of the seedlings in both zones were similar (Table 3.4). Biomass partitioning of *A. alba* seedlings in both intertidal zones were consistent with the results obtained from the field experiment (Table 3.4). RGR (Figure 3.7) was not affected by the tidal inundation during the first three months ($t_{2(34)}$ = 0.44, p = 0.658). However, at the end of the study period, a significant difference in biomass partitioning (Table 3.4) and RGR ($t_{2(34)}$ = 2.22, p = 0.020) was observed in *A. alba* seedlings at the two intertidal zones.

Table 3.4. Biomass allocations of *A. alba* seedlings at two intertidal zones in the field (n = 36)

Biomass	Intertidal zone	Time (months)		
		0	3	6
Total (g)	**UI**	0.53 ± 0.10 [a]	0.97 ± 0.15 [a]	1.83 ± 0.18 [a]
	LI	0.49 ± 0.06 [a]	0.94 ± 0.13 [a]	1.49 ± 0.12 [b]
Root (g)	**UI**	0.22 ± 0.04 [a]	0.42 ± 0.09 [a]	0.74 ± 0.06 [a]
	LI	0.20 ± 0.03 [a]	0.42 ± 0.07 [a]	0.65 ± 0.06 [b]
Shoot (g)	**UI**	0.31 ± 0.07 [a]	0.55 ± 0.10 [a]	1.09 ± 0.14 [a]
	LI	0.29 ± 0.04 [a]	0.53 ± 0.07 [a]	0.83 ± 0.10 [b]
R/S	**UI**	0.70 ± 0.09 [a]	0.78 ± 0.17 [a]	0.69 ± 0.07 [a]
	LI	0.69 ± 0.08 [a]	0.79 ± 0.09 [a]	0.79 ± 0.11 [b]

Mean and standard deviation, means in the same column with different letters in the same column are significantly different at α 0.05

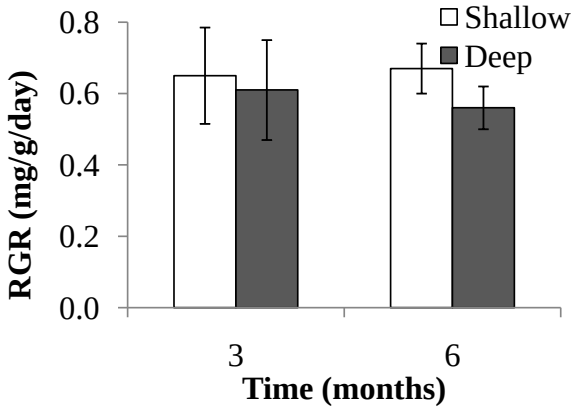


Figure 3.7. Relative growth rates of *A. alba* seedlings in two intertidal zones (n = 36)

3.3.3.3 Soil physical parameters

Results of soil particle size distribution showed no significant differences in soil texture in both intertidal zones. Similarly, there was no significant difference in pH of the soil in both zones ($t_{2(34)}$ = 1.68, p = 0.102).

3.4 Discussion

3.4.1 Root development and establishment of propagules

All the propagules developed roots irrespective of the tidal treatment in the greenhouse. However, deep treatment facilitated rapid root development in *A. alba* (Figure 3.2). In most terrestrial plants, water availability breaks seed dormancy and facilitates germination

(Akhalkatsi and Lösch, 2005; Bradford, 1994). However, unlike most plants, mangrove trees produce viviparous propagules whose seeds germinate while still attached to the maternal tree (Tomlinson, 1986). In addition, mangrove species of the genus *Avicennia* produce comparatively smaller propagules than *Rhizophora* and *Bruguiera*. The propagules of *A. alba* with small rootlets is observed floating in large numbers on coastal mangrove strands where this species prevail, suggesting that these propagules develop rootlets before they reach a suitable habitat. Hence, tidal inundation does not affect root development of *A. alba* propagules.

In contrast to root development, the sea level treatments had profound effects on the establishment of *A. alba* seedlings (Figure 3.3). Percentage establishment decreased significantly in the deep treatment. In a similar study, propagule establishment of *K. candel* and *B. gymnorrhiza* was not affected by the tidal inundation (Ye et al., 2003). Variations in the ability to establish under different water levels is a key governing factor of the zonation patterns observed in the mangrove forests where different species predominate along the intertidal zones (Urrego et al., 2009). Apart from physical environmental factors, morphological characteristics such as size and buoyancy of the propagules affect their establishment (Clarke et al., 2001; Rabinowitz, 1978).

3.4.2 Seedling growth and survival

Effects of prolonged waterlogging and tidal inundation on seedling growth indicate varying responses in different species (Delgado et al., 2001; Ellison and Farnsworth, 1997). Tidal inundations at the intertidal zones and the flooding treatments in the greenhouse had profound effects on the stem height of *A. alba* seedlings. Both LI zone and deep treatment

facilitated stem height increment in *A. alba* during the first few months (Figures 3.4 and 3.6). Similar results have been reported in *Rhizophora mangle* (Ellison and Farnsworth, 1997), *Bruguiera gymnorrhiza* and *Kandelia candel* (Ye et al., 2003) and *Laguncularia racemosa* (Delgado et al., 2001). Stem elongation in response to flooding enables the shoot to remain above the water surface for a longer period and thus sustain aerial respiration and photosynthesis and minimize the stress associated with soil reduction (Das and Uchimiya, 2002). In contrast, high elevation decreased stem height of *Rhizophora apiculata* (Kitaya et al., 2002a) and tidal flooding did not affect stem height of *Avicennia germinans* (Delgado et al., 2001) suggesting distinct effects of tidal inundation on different species.

High sea level in LI zone and deep treatment decreased stem diameter and number of new leaves (Table 3.1 and 3.3) of *A. alba* towards the end of the experiment. Prolonged tidal inundation increased leaf fall in the greenhouse and in the field (Table 3.1 and 3.3). In a comparative study on the effects of stimulated sea level rise on *B. gymnorrhiza* and *K. candel*; Ye et al., (2004) reported similar growth responses in *B. gymnorrhiza* and concluded that it is a less tolerant species to sea level rise. These similarities in the growth responses of *A. alba* and *B. gymnorrhiza* under prolonged tidal inundation could explain the distribution of these two species in east Malaysia, where both species coexist in the coastal zones at low to medium elevations (Satyanarayana et al., 2010). Several mangrove plants respond to prolonged inundation by altering the physiological processes such as stomatal closure, reduced water uptake, transpiration rate and photosynthetic rate causing stunted growth (Naidoo, 1985; Pezeshki et al., 1997). Reduced leaf size have been reported in *L. racemosa* and *R. mangle* (Pezeshki et al., 1990b) under prolonged tidal inundation. Growth

of *A. alba* seedling in this study suggests that sea level rise of 30 cm may not affect very early growth but inhibit growth afterwards (Ellison and Farnsworth, 1997; Ye et al., 2004).

Tidal inundation in the LI zone affected survival of *A. alba* seedlings. At the end of the study period, survival of *A. alba* seedlings in the LI zone was significantly lower than those in the UI zone; however in the greenhouse the difference was not significant (Figures 3.4 and 3.6). Differences in tidal inundations resulting from elevations in the coastal environment affect seedling growth and survival (He et al., 2007; Kitaya et al., 2002a). Seedling mortality at lower intertidal zones is often attributed to the harsh environmental conditions such as greater tidal actions and sedimentation (Krauss et al., 2008). Despite these conditions several mangrove species thrive in lower intertidal zones where prolonged tidal inundation prevail indicating different species may differ in their response to tidal inundation (Youssef and Saenger, 1996). Nevertheless, at the seedling stage, the effects of tidal inundation is more severe and the stress associated with prolonged inundation can lead to weaker seedlings and reduce survival rates in species that are less tolerant to tidal inundation (Pezeshki et al., 1990a) as observed in *A. alba* in this study.

3.4.3 Biomass allocations and RGR

Total biomass (Table 3.2 and 3.4) and RGR (Figures 3.5 and 3.7) of *A. alba* seedlings in the LI zone and in the deep treatment in greenhouse indicated significant reductions towards the end of the experiment. Similar results have been reported for *A. germinans* (Pezeshki et al., 1997), *R. mangle* (Pezeshki et al., 1990b) and *B. gymnorrhiza* (Krauss and Allen, 2003; Ye et al., 2003). The major consequence of tidal flooding is restriction in soil-atmospheric gas exchange which decreases soil Eh (Pezeshki, 2001). Soil reduction and subsequent soil

physicochemical changes cause a wide range of stress symptoms in mangrove plants (Kozlowski, 1984a; Perata and Alpi, 1993). The inherent ability to withstand abiotic stress is often attained at the cost of reduced photosynthesis resulting from limited gas exchange due to stomatal closure and metabolic inhibition (Pezeshki, 1993).

Reduced growth potential of seedlings under tidal inundation could occur from whole-seedling biomass reallocation (Krauss et al., 2006a). *A. alba* seedlings in the current study showed a higher R/S ratio in the LI zone in the field and in the deep treatment in the greenhouse during the last three months. Shifts in biomass allocation from root to shoot in *K. candel* have been reported as an adaption to shorten the oxygen diffusion path to the growing root tips (Ye et al., 2003, 2004). Alternatively, seedlings could invest more biomass to produce above ground roots that facilitate gas exchange as observed in species within the family Rhizophoraceae (Yanez-Espinosa et al., 2001).

In this study, the increased root biomass of *A. alba* seedlings in the LI zone and in the deep treatment is attributed, firstly to the decreased leaf production and increased leaf senescence. Decreased leaf size and leaf biomass have been reported in several mangrove species subjected to prolonged tidal inundation (Pezeshki et al., 1990b; Ye et al., 2004). Moreover, several plants accumulate abscisic acid (ABA) in response to stresses which gives rise to a number of growth responses in plants (Chen et al., 2004). Luzhen et al., (2005) reported increased levels of ABA in the mature leaves of *K. candel* seedlings under prolonged tidal inundation. Apart from decreased photosynthesis and gas exchange as a result of stomatal closure; ABA induced leaf senescence accounting for decreased shoot biomass (Kozlowski, 1984a) which further reduces photosynthesis in plants under tidal inundation.

Secondly, several individuals of *A. alba* grown in the LI zone and in the deep treatment in the greenhouse developed pneumatophores. Development of pneumatophores plays a significant role in facilitating gas exchange and minimizes stress associated with soil reduction (Kitaya et al., 2002b). In the natural settings at high tide pneumatophores aid gas exchange by the removal of respiratory CO_2 and generate low pressure within these structures that result in an influx of gas through the exposed shoot; and at low tide rapid influx of air aids recovery from stresses caused by the limited gas exchange (Skelton and Allaway, 1996). Furthermore, pneumatophores of *A. marina* and *S. alba* conduct photosynthesis, generating oxygen within these structures that facilitate oxygen uptake at high tide (Kitaya et al., 2002b). However, allocation of biomass to roots relative to shoots require a significant proportion of fixed carbon in mangrove seedlings (McKee, 1996). Hence, mangrove seedlings may trade high growth rates for allocation of biomass to aboveground roots as a strategy to tolerate tidal inundation (Cardona-Olarte et al., 2006) as evident in *A. alba* seedlings in the present study.

3.4.4 Soil physical parameters

Treatments in the greenhouse altered soil physical characteristics. Prolonged waterlogging in the greenhouse decreased soil Eh and pH significantly. Profound effects of flooding on soil physicochemical characteristics are well documented (Narteh and Sahrawat, 1999; Ponnamperuma, 1972; Unger et al., 2009). Under tidal inundation, available oxygen in the soil is reduced by the roots, microorganisms and soil reductants (Ponnamperuma, 1984). The subsequent chemical changes include denitrification and reduction of mineral ions (Gambrell and Patrick, 1978) causing Eh more negative. Furthermore, pH is lowered as

microbial metabolic waste accumulates in the soil (Alef, 1995). These physicochemical changes in the soil alter the oxygen demand in the root medium which is a key regulating factor in wetland functions (Delaune and Pezeshki, 1991) and community structure (Armstrong et al. 1985)

In experimental cultures, oxygen supply in the soil can be reduced rapidly as much as 28% after 6 hours of flooding (Skelton and Allaway, 1996). The intensity of reduction depends on soil characteristics such as soil texture (Pezeshki, 2001). Ye at al., (2004) showed that the acidity due to water level rise was more intense in fine soil than in course soil. Despite slow growth and survival of *A. alba* seedlings in the LI zone, no significant differences in soil texture were observed at the intertidal zones. Similarly, variations in the soil pH in both intertidal zones were not significant.

Several plant responses to flooded soil conditions have been reported (Drew, 1992; Vartapetian and Jackson, 1997). Correlation analyses of the soil physical parameters and growth of *A. alba* suggest reduced growth and increased biomass allocation to the roots under decreased soil Eh and pH. These results further suggest that unlike *K. candel* (Ye et al., 2003), *A. alba* invests in the development of pneumatophores, demonstrating the differential strategies exhibited by the individual species to overcome the problems associated with soil reduction and decreased pH.

3.5 CONCLUSION

The effects of prolonged tidal inundation under simulated sea levels in the greenhouse and in the field indicated stunted growth, decreased growth rates and survival of *A. alba*

seedlings under sea level rise. These results suggest *A. alba* seedlings could be less tolerant to sea level rise. However, *A. alba* seedlings in the deep treatment in the greenhouse and in the LI zone in the field developed pneumatophores as an adaptive strategy to facilitate gas exchange and to minimize the effects of oxygen deficiency in the soil. Such adaptive strategies exhibited by the individual species to overcome the stresses associated with the rising sea levels provide crucial information on the distinct responses of the species to prolonged tidal inundation. However the extent of the development of pneumatophores and the ability to reach maturity under tidal inundations needs further experimental evaluation.

CHAPTER 4 - PHYSIOLOGICAL RESPONSES AND CARBON SEQUESTRATION OF *AVICENNIA ALBA UNDER SIMULATED SEA LEVEL RISE*

4.1 INTRODUCTION

Mangroves possess a variety of morphological and physiological adaptations that enable them to occupy the intertidal zones along the tropical and subtropical coastlines (Ball, 1988b). At the intertidal zones, these plants are exposed to various degrees of stresses resulting from hyper saline substrates, soil anoxia from tidal flooding, extreme irradiance and, strong winds and currents (Duke et al., 1998a). In spite of these stresses, mangroves thrive under these harsh conditions and provide valuable site specific ecological and socioeconomic values.

Naturally, mangrove communities encounter altered flood depths and durations resulting from tide fluctuations, sea level rise (Ellison and Stoddart, 1991; Woodroffe, 1987) and human-induced regional hydrologic changes (Choy and Booth, 1994; Elster, 2000). The distinct zonations observed in the mangrove forests indicate that different species may exhibit different tolerance to tidal inundations. The differential abilities to cope with tidal inundation could be attained through a combination of morphological, anatomical, physiological or genetic adaptations (Kozlowski, 1984b; Youssef and Saenger, 1996).

The physiological variations among mangrove plants have been well documented (Ball, 1988b; Lovelock et al., 2006). Mangrove are classified as stress tolerant and are able to respond to variations in the abiotic environment through altered biochemical processes via hormone mediated growth constraints or by decreasing resource acquisition (Chapin, 1991).

However, in some regions, mangrove trees export substantial amounts of organic carbon which makes the mangrove ecosystems extremely productive wetlands (Saenger, 2002).

The early growth of *A. alba* under simulated sea level rise indicated reduced growth and relative growth rates under prolonged tidal inundation (Chapter 3). Thus, the aims of this study are, firstly, to study the physiological responses of *A. alba* under tidal treatments; and secondly, to determine carbon sequestration in *A. alba* at a range of CO_2 concentrations under different tide treatments. It is anticipated that the ability to enhance assimilation with increasing CO_2 concentrations under tidal flooding is a promising feature that will increase the population of *A. alba* in the context of the current climate change.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design and Culture Conditions

The experimental setup of simulated sea level rise in the greenhouse was the same as that described in section 3.3.1.2 with two tidal treatments; deep and shallow. The seedlings were cultured according to the methods stated in section 3.3.1.3.

4.2.2 Physiology and Carbon Sequestration

Data collection was carried out on the third and sixth month after subjecting the seedlings to the treatments. Data was obtained between 10 am and 12 pm when stomatal conductance of the seedling was predetermined to be the highest. Six seedlings per treatment were randomly chosen to study the physiological responses and carbon sequestration of *A. alba* seedlings under simulated sea level rise. A portable photosynthesis system (PPSystem,

CIRAS-1, USA) was used to obtain both photosynthetic and carbon sequestration data on *A. alba* seedlings (Plate 4.1) Measurements were recorded by clipping 2.5 cm^2 of sample from the youngest fully expanded leaves to a microclimate chamber of the photosynthesis system.

Plate 4.1. Measurement of physiological parameters using the portable photosynthesis system

Rate of assimilation (A) and stomatal conductance (g_s) under ambient conditions in the greenhouse were recorded to determine the effects of tidal treatment on physiological responses of *A. alba*. Chlorophyll conductance of *A. alba* seedlings in both treatments were recorded using a portable chlorophyll meter (SPAD-502, Minolta, Japan) to determine if the

variations in the physiological responses of *A. alba* were related to chlorophyll conductance of the plants which could be altered as a result of the tidal treatments.

Carbon sink potential was studied by supplying different CO_2 concentrations ranging from 0 – 1500 ppm, by a pure CO_2 canister (Sparklets, model E290, Solengen, Germany) mixed with CO_2 free air at a Photosynthetically Active Radiation (PAR) of 1800 μmol m^{-2} s^{-1}. Rate of assimilation (A), stomatal conductance (g_s) and transpiration (E) at different CO_2 concentration (C_a) was recorded simultaneously. Maximum photosynthetic rates (A_{max}) at saturated CO_2 and photosynthetic rates at carbon dioxide concentration of 400 ppm (A_{400}) were estimated from the CO_2 response curves (photosynthetic rates (A) *vs.* C_a curves) by a non-linear regression to hyperbolic equation. CO_2 compensation point (Γ_{CO_2}) was estimated at assimilation (A) = 0.

4.2.3 Data Analyses

Independent sample t-test was performed to test any differences between tidal treatments in the ambient physiological parameters, chlorophyll conductance and carbon sequestration with STATISTICA version 9.0 (Statsoft Inc).

4.3 RESULTS

Sea level treatments affected assimilation (A) and stomatal conductance (g_s) of *A. alba* seedlings (Figure 4.1 and 4.2). At third month, assimilation of *A. alba* seedlings in the deep was 52% less than that of shallow treatment while assimilation at sixth month was 47% lower in the deep treatment. A significant decrease in A was observed in the deep treatment

at third month ($t_{2(10)}$ = 3.88, p = 0.003) and sixth month ($t_{2(10)}$ = 5.14, p = <0.001). Stomatal conductance (g_s) of *A. alba* seedlings at ambience varied with time. A 50% decrease in g_s was observed in the deep treatment at third month which was significantly lower than that of shallow treatment ($t_{2(10)}$ = 3.65, p = 0.004) while at sixth month, g_s of *A. alba* seedlings in the deep treatment was reduced by 28%, but this difference was not significant ($t_{2(10)}$ = 1.99, p = 0.075). Chlorophyll conductance of *A. alba* seedlings (Figure 4.3) were not affected by the sea level treatments ($t_{2(34)}$ = 1.77, p = 0.085). However the average chlorophyll conductance of the seedlings in the deep treatment was slightly higher than that of the shallow treatment.

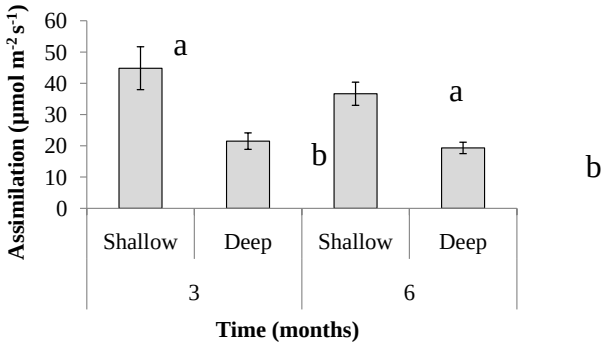


Figure 4.1. Assimilation rate of *A. alba* at ambience in two treatments at both third and sixth month (n = 12). Means at each time interval with different letters are significant at α 0.05.

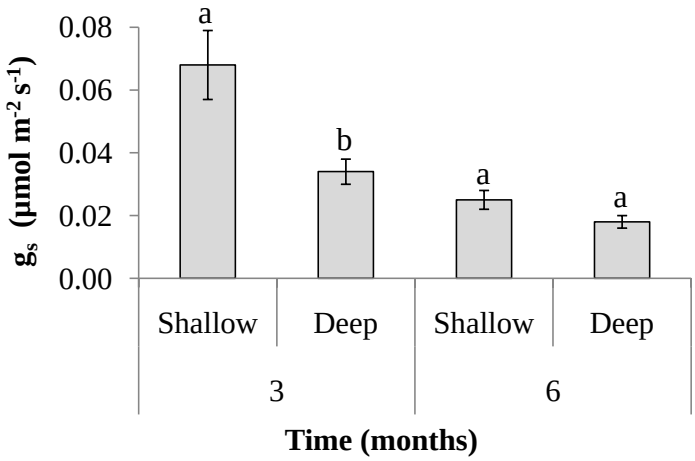


Figure 4.2. Stomatal conductance at ambience (g_s) of *A. alba* in two treatments at both third and sixth months (n = 12). Means at each time interval with different letters are significant at $\alpha = 0.05$.

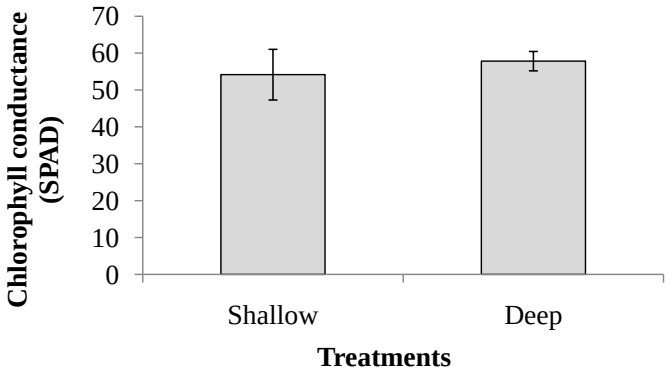


Figure 4.3. Chlorophyll conductance of *A. alba* in two treatments at sixth month (n = 36)

Sea level treatments had profound effects on carbon sequestration in *A. alba* seedlings (Table 4.1). At third month, A_{400} of *A. alba* seedlings in the deep treatment was 60.3% lower than those in the shallow treatment. Likewise, A_{max} and g_s of the seedlings in the deep

treatment was reduced by 59.6% and 26.9% respectively, compared to that of the shallow treatment. The average transpiration rate (E) was reduced by 74.87% in the deep treatment.

At sixth month, the difference in photosynthetic components between the two treatments was less variable. A_{400} in the deep treatments at sixth month was 34.7% lower than those in the shallow treatment. Similarly, the difference in A_{max} and g_s were 26.74 and 54.17% respectively, lower than shallow treatment. The decrease in the transpiration rate in the deep treatment was 36.59%. However, a significant difference between the tidal treatments was observed only in A_{max} and A_{400}.

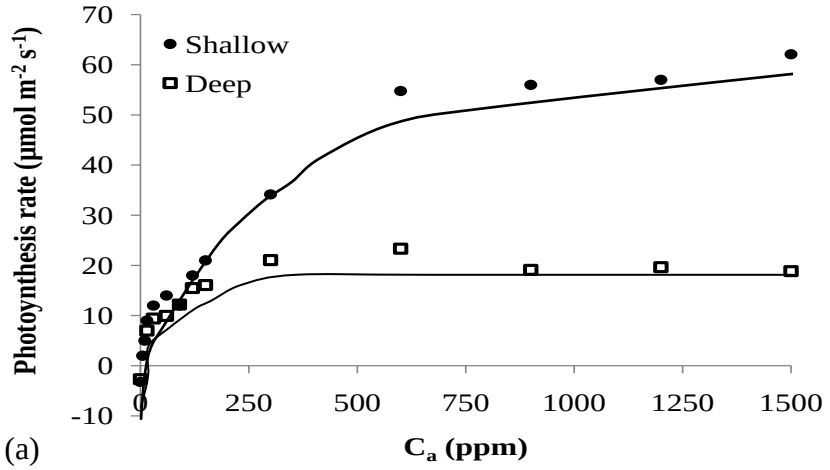
Table 4.1. Photosynthetic components of *A. alba* in the deep and shallow treatments at third and sixth month (n = 12)

Photosynthesis component	**Third month**			**Sixth month**		
	Deep	**Shallow**	**t-statistic**	**Deep**	**Shallow**	**t-statistic**
A_{max} (µmol m^{-2} s^{-1})	23.3	58.7	4.73***	6.2	9.5	2.50*
A_{400} (µmol m^{-2} s^{-1})	18.4	45.6	5.42***	5.2	7.1	2.53*
g_s (µmol m^{-2} s^{-1})	0.019	0.026	3.06*	0.011	0.024	1.39
E (µmol m^{-2} s^{-1})	0.51	2.03	2.41*	0.52	0.82	1.77
Γ_{CO2} (ppm)	37.6	35.4	0.33	34.7	34.9	0.02

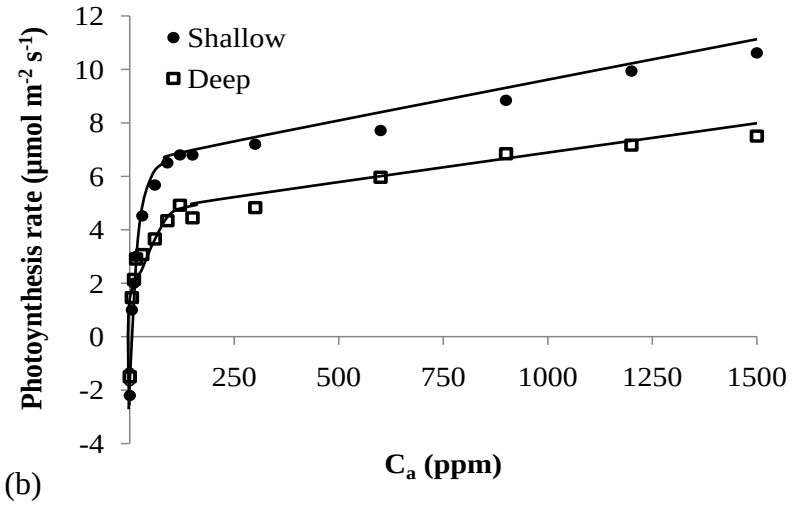
Means and standard deviations of six replicates are shown. Significant variation in t-statistics between treatments is indicated as α: * < 0.05, ** < 0.01 and *** < 0.001

At third month, *A. alba* seedlings in the shallow treatment increased photosynthetic rates with increasing C_a and CO_2 saturation was observed at 500 ppm (Figure 4.4 (a)). In contrast, in the deep treatment, CO_2 saturation was observed below ambience at 175 ppm and further increases in C_a did not increase the rate of photosynthesis. The variation in CO_2 response curves at sixth month was less variable between tidal treatments and CO_2 saturation was

observed below ambience in both treatments (Figure 4.4 (b)), however, in both treatments, photosynthetic rate gradually increased with increasing C_a.



(a)



(b)

Figure 4.4. CO_2 response curves of *A. alba* in two treatments at PAR 1800 µmol m^{-2} s^{-1} at (a) 3rd and (b) 6th month (n = 12).

The relationship between g_s and C_a of *A. alba* in both treatments showed a similar trend during the third month (Figure 4.5 (a)). In both treatments, g_s increased linearly to a maximum and decreased gradually with increasing C_a. Across all the carbon dioxide concentrations g_s of *A. alba* seedlings in the shallow treatment was higher than those in the deep treatment. At sixth month, g_s in the shallow treatment remained constant across all C_a concentrations whereas in the deep treatment it decreased rapidly with increasing C_a. (Figure 4.5 (b)).

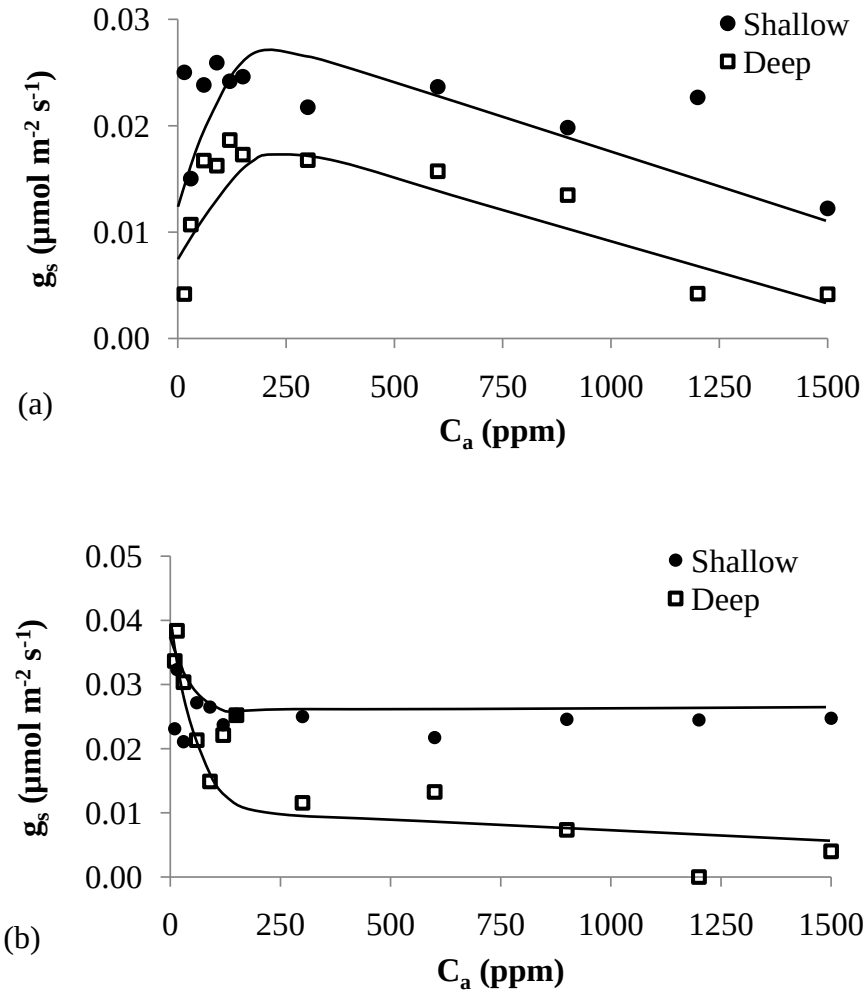


Figure 4.5. Relationship between stomatal conductance and C_a of *A. alba* seedlings in two treatments at (a) 3rd and (b) 6th month (n = 12).

The relationship between transpiration rate (E) and C_a of *A. alba* in the shallow treatment was the same at third and sixth month where E was not affected by the increasing C_a (Figure 4.6). However, in the deep treatment, at third month, E increased with increasing C_a up to 175 ppm and decreased rapidly with further increase in C_a (Figure 4.6 (a)). In contrast, at sixth month, E decreased rapidly with increased C_a (Figure 4.6 (b)).

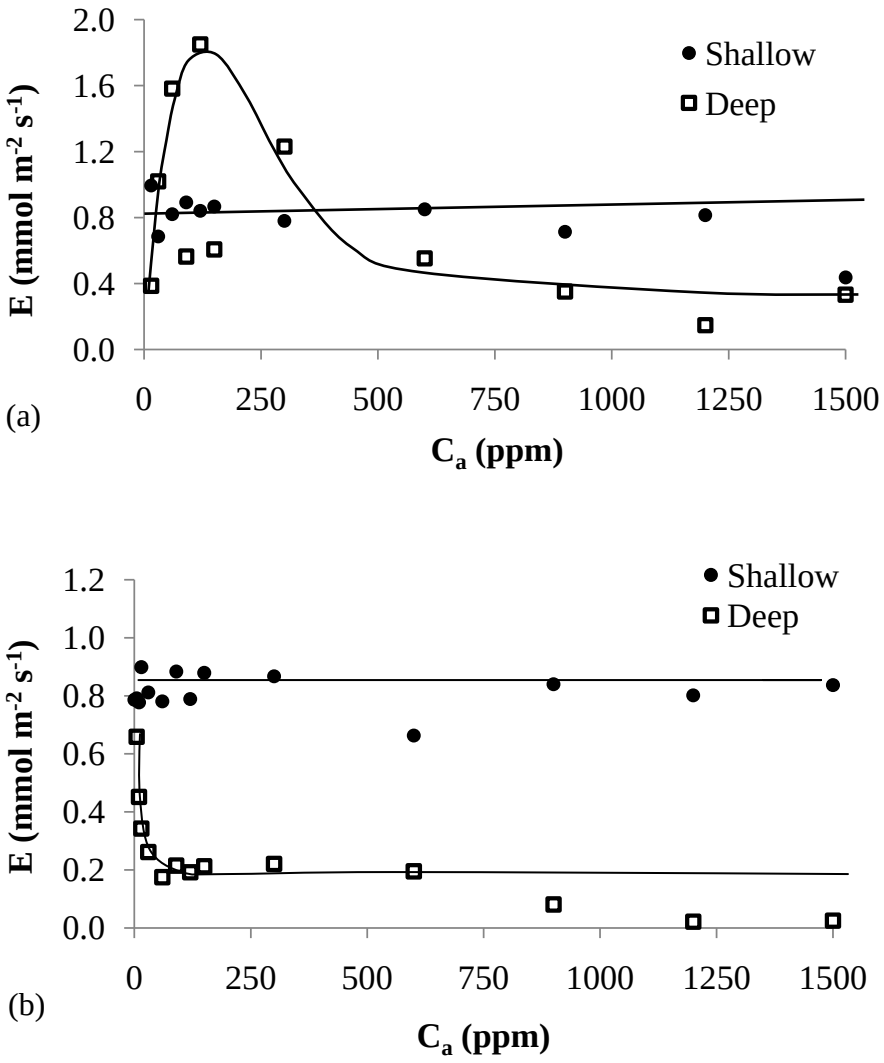


Figure 4.6. Relationship between transpiration rate and C_a of *A. alba* seedlings in two treatments at (a) 3rd and (b) 6th month (n = 12)

4.4 DISCUSSION

Assimilation of *A. alba* in the greenhouse under ambience showed significant differences resulting from tidal treatments (Figure 4.1). At both time intervals, decreased assimilation was observed in the deep treatment. Several experiments on tidal flooding and physiological responses of mangrove species indicate reduced assimilation under prolonged tidal inundation. Tidal flooding reduced assimilation in *R. mangle* (Ellison and Farnsworth, 1997) and *K. candel* (Chen et al., 2005). Similarly, short term flooding of 6-22 days decreased leaf-level carbon assimilation by 20% in *A. germinans*, *L. racemosa* and *R. mangle* (Krauss et al., 2006b). In the same study, long term flooding was found to have slight effects on the leaf gas exchange characteristics of mangrove seedlings.

In general, decreased assimilation under prolonged tidal flooding is attributed to stomatal closure (Kozlowski, 1984b; Pezeshki, 1994) resulting from changes in the guard cell turgor or enhanced root resistance to water uptake (Naidoo et al., 1997). Stomatal conductance of *A. alba* at ambience reduced significantly in the deep treatment but the difference was less at sixth month (Figure 4.2). Decreased stomatal conductance was observed in the seedlings of *A. marina* when subjected to short periods of tidal inundation (Sayed, 1995). Prolonged tidal inundation induced increased stomatal resistance and decreased water potential in *B. gymnorrhiza* (Naidoo, 1983). Similarly, stomatal closure in *K. candel* caused reductions in the intercellular CO_2 concentrations inhibiting assimilation and transpiration. In addition to stomatal closure, stomatal density was also reduced in *K. candel* with prolonged tidal inundation (Ye et al., 2004). However, the range of g_s was less variable under tidal flooding among saplings compared to the seedlings indicating a time dependent shift in stomatal

adjustments in mangrove plants (Krauss et al., 2006b). Rapid recovery in g_s was observed in *A. marina* seedlings when simulated high tides were removed (Sayed, 1995). Similarly, stomatal reopening could occur in flood-tolerant species under long-term flooding (Pezeshki, 1993). These findings suggest that mangroves may develop tolerance to tidal inundation with prolonged exposure or with plant age and that recovery from prolonged inundation is probable in certain species.

Chlorophyll conductance of *A. alba* was not significantly different between tidal treatments (Figure 4.3) suggesting that assimilation in the deep treatment was not limited by the folia chlorophyll conductance. However, deep flooding increased the amount of total chlorophyll in *B. gymnorrhiza* and *K. candel* (Ye et al., 2003, 2004) but this increase in photosynthetic pigments did not increase the relative growth rates of either species under prolonged inundation (Ye et al., 2003). In contrast, simulated sea level rise of 16 cm decreased the amount of chlorophyll in *R. mangle* which was associated with decreased net assimilation rate, relative growth rates and A_{max} (Ellison and Farnsworth, 1997). Thus, tidal flooding may vary chlorophyll conductance in mangrove species depending on the capacity of individual species to tolerate prolonged inundation.

Carbon sequestration of *A. alba* at third month indicated higher carbon sink potential under shallow treatment where CO_2 saturation was observed at 500 ppm whereas in the deep treatment it was 175 ppm which is below ambience (Figure 4.4 (a)). In a 2 ½ year study on *R. mangle* under simulated sea level rise, Ellison and Farnsworth (1997), reported that 10-25% reduction in plant growth characteristics under simulated sea level rise and led to the conclusion that any positive effects of increased atmospheric CO_2 might be compensated by reduced growth in response to longer hydroperiods and deeper tidal inundations. The

variation in CO_2 response curves at sixth month was less variable between tidal treatments (Figure 4.4 (b)) and in both treatments, CO_2 saturation was observed below ambience. However, in both treatments, increasing C_a increased photosynthesis suggesting that prolonged exposure to tidal flooding increased the carbon sequestration capacity over a range of C_a. The results of both physiological responses and carbon sequestration was consistent where the effects of tidal treatments affected physiology and carbon sequestration more at third month but the differences were hindered at sixth month suggesting that mangrove may become less sensitive to tidal flooding with the duration of the exposure (Krauss et al., 2006b) through physiological adaptations.

The relationship between g_s and C_a was prominent during the third month (Figure 4.5 (a)). In both treatments, increasing C_a increased g_s to a maximum and further increase in C_a decreased g_s rapidly. At sixth month increase in C_a did not affect g_s in the shallow treatment but rapid decline in g_s was observed in the deep treatment (Figure 4.5 (b)). Decreased g_s with increasing C_a have been reported in plants under stress (Drake et al., 1997; Morison, 1993) resulting from increased stomatal sensitivity to stresses under elevated CO_2 (Tognetti et al., 2000). The observed trend in g_s in the deep treatment is consistent with reduced assimilation rates regardless of C_a and could result from stomatal adjustment in response to tidal flooding. A similar response is observed between E and C_a (Figure 4.6) indicating that the rate of transpiration is consistent with stomatal aperture. Thus, stomatal closure under elevated CO_2 is an adaptive strategy that allows optimum assimilation with minimal waterloss through transpiration (Jackson et al., 1994).

4.5 CONCLUSION

Tidal flooding decreased both assimilation rate and stomatal conductance of *A. alba*. Reduced assimilation is attributed to stomatal adjustment under simulated sea level rise. Similarly, assimilation in *A. alba* in the deep treatment was not limited by the chlorophyll conductance of the seedlings as there were no significant differences in the chlorophyll conductance between the tidal treatments. Similarly, carbon sequestration was affected where both A_{400} and A_{max} decreased in the deep treatment. The results of the CO_2 response curves showed decreased assimilation in *A. alba* regardless of the CO_2 concentration under tidal flooding at third month suggesting that *A. alba* did not respond to elevated CO_2 under tidal flooding. Thus, the benefits of increased atmospheric CO_2 could be offset by the stresses associated with rising sea levels in *A. alba* at the seedling stage. However, at sixth month, increased C_a increased photosynthetic rates but carbon dioxide saturation occurred below ambience in both treatments. Similarly, less variation in g_s and E was observed at sixth month indicating that *A. alba* seedlings exhibited some adaptations to prolonged tidal inundation over time.

CHAPTER 5 - ECOLOGICAL RESPONSES OF *AVICENNIA MARINA VAR. ACUTISSIMA AND BRUGUIERA PARVIFLORA UNDER SIMULATED SEA LEVEL RISE*

5.1 INTRODUCTION

The distinct zonation patterns in the mangrove forests correlate with the frequency and duration of tidal flooding (Macnae, 1968; Watson, 1928). Tide level influence factors such as soil saturation, nutrient availability and the salinity of surface and soil water (Pezeshki et al., 1990a). These factors affect growth and resource availability, thus influence vegetation of the mangrove forests (Pezeshki, 2001). Generally, the species that are more tolerant to high salinities and prolonged tidal flooding occupy the lower inter tidal zones while those species that are more sensitive to abiotic stresses are found closer to the landward margins of the forests (He et al., 2007).

Several studies, under simulation in the greenhouse and in the field have been the focus of recent literature to assess the differential tolerance among mangrove species under different environmental gradients (Ellison and Farnsworth, 1997; Kitaya et al., 2002a; Ye et al., 2010). With the ongoing changes in the climate, projected sea level rise pose a significant threat to mangrove ecosystems (Alongi, 2008; Ellison and Stoddart, 1991; Gilman et al., 2008). Prolonged tidal inundation associated with rising sea levels is likely to cause intense soil anoxia and increased toxins in the soil that induce various degrees of growth constraints in plants (Pezeshki, 2001). However, sensitivity to such stresses vary between species and several studies indicate differential tolerance to prolonged tidal inundations in mangrove species (Pezeshki et al., 1990b).

The effects of tidal inundation on mangrove seedlings have been reported for several species (Krauss et al., 2008). Prolonged tidal inundation in the greenhouse induced lower height, stem diameter and biomass in *Bruguiera gymnorrhiza* (Krauss and Allen, 2003). Likewise, flooding reduced leaf size in *Laguncularia racemosa* (Pezeshki et al., 1990b). Tidal flooding reduced the relative growth rates of *B. gymnorrhiza* but did not affect growth of *Kandelia candel* (Ye et al., 2003) and deep flooding and longer hydroperiods simulated growth of *R. mangle* indicative of lower intertidal positions (Ellison and Farnsworth, 1993).

In Malaysia, *Avicennia marina* var. *acutissima* and *B. parviflora* are common occupants of the mangrove forests. The distributions of these species vary along the intertidal zone, where *A. marina* occupies the lower intertidal zones while *B. parviflora* is more abundant in the landward margin of the forests. Thus, it is hypothesized that these two species will have differential tolerance to tidal inundation where *A. marina* is better adapted to tidal inundation than *B. parviflora*. The aim of this study is to study and compare ecological responses of *A. marina* var. *acutissima* and *B. parviflora* under simulated sea level rise.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Design

Two identical fibreglass tanks measuring 226 cm long x 122 cm wide x 76 cm deep were used to setup artificial tidal tanks simulating daily semidiurnal tides with two “high” and “low” tide periods. Artificial seawater was prepared by dissolving commercial sea salt and the salinity was maintained at 30 ‰ throughout the study period either by adding water or salt whenever needed. In each tidal tank, four subplots were established.

Two treatments, shallow and deep were specified and each tank represented one treatment. The shallow treatment mimicked the current tidal fluctuations with daily 12 hours of high and low tides. Seawater was pumped into the shallow tanks at “high tide” at 00:00 hrs and 12:00 hrs and ebbed at “low tide” at 06:00 and 18:00 hrs. During high tide the water level was 20 cm and at low tide water level was 5 cm.

The deep treatment tank simulated tidal fluctuations under sea level rise with 18 and 6 hours of daily high and low tide periods respectively. In this tank, sea water was pumped into the tanks at “high tide” from 01:00 to 09:00 and 13:00 to 21:00 and ebbed at “low tide” from 09:00 to 13:00 and 21:00 to 01:00. Water level of high and low tide was 50 and 20 cm respectively (Plate 5.1). All the seedlings in the deep treatment were fully submerged at high tide at the beginning of the experiment.

Plate 5.1. Deep treatment tank at high tide at the end of the study period

5.2.2 Seedling Growth

Healthy, mature propagules of *A. marina* var. *acutissima* and *B. parviflora* were collected from a mangrove forest in Klang River, Malaysia. The propagules were planted individually in polybags (10 x 20 cm) using soil from the mangrove forest as a potting medium. In the previous study, survival of *A. alba* seedlings was highly reduced when harvested and transplanted (Chapter 3). In order to overcome high mortality rates, in this study, the seedlings were nurtured in a nursery adjacent to the mangrove forest in the Klang River under natural tidal conditions for a period of three months. The average initial height of the seedlings of *A. marina* and *B. parviflora* ranged between 39 ± 2.7 and 19.5 ± 3.5 cm (mean ± standard deviation) respectively. The seedlings were acclimatized in the greenhouse for five days before subjecting to the treatments. At the onset of the experiment, 10 seedlings of each species were placed in each of the subplots and were monitored for a period of six months.

The initial data on height, diameter at the first stem node, number of leaves and leaf fall were recorded. The consecutive data on these parameters were obtained at monthly intervals. Diameter at first stem node was recorded to the nearest 0.01mm using a digital calliper. The total increase in leaf number was defined as double the number of stem nodes because both species produce opposite leaves with two leaves at each stem node. Leaf fall was determined from the difference between the number of stem nodes and the number of leaves remained on the plant.

5.2.3 Biomass Allocation and RGR

At the beginning of the experiment, 20 seedlings from each species were used to determine the initial biomasses of the seedlings. The consecutive data on biomasses, biomass allocations, root to shoot ratio and relative growth rates (RGR) were determined every three months according to the methods described in section 3.3.1.4. Five seedlings of each species from each plot (20 seedlings from each treatment, n = 40 per species) were harvested randomly to collect consequent data on biomass and RGR at these time intervals.

5.2.4 Soil Physical Parameters

At the onset of the experiment, 40 pots of each species were randomly sampled to determine the initial soil pH. Variations in soil pH and redox potential (Eh) resulting from different tidal treatments in each species were obtained at the end of the study period. Similar methods as described in section 3.3.15 were employed.

5.2.5 Data Analysis

As the majority of the growth parameters recorded varied greatly between both species, the statistical significances of these parameters were conducted within tidal treatments of the same species. The effects of deep and shallow treatments on growth parameters at monthly intervals were tested by the independent sample t-test. Furthermore, to test if the growth responses of the seedlings were affected by the treatment and the duration of the exposure to the treatments, two-way repeated measures of ANOVA was performed with treatment and time (six months) as the within-subject factors using 20 random data points for each

species in each treatment for each month. Two-way ANOVA was performed to test if the variations in soil physical characteristics varied between treatments and species. For each species correlation analyses were performed to determine the relationship between soil physical characteristics and relative growth rates and r/s ratio of each species at the end of the study period. All the analyses were performed with SPSS version 16.0 software.

5.3 RESULTS

5.3.1 Seedling Growth

Simulated sea level rise induced stem elongation in both species. Stem height of *A. marina* in the deep treatment was significantly higher throughout the study period (Table 5.1). Deep treatment also induced stem elongation in *B. parviflora* but no significant differences were observed during the last two months (Table 5.1). In contrast, stem diameter was reduced in the deep treatment in both species. *A. marina* had significant reductions in the stem diameter during the last two months and stem diameter of *B. parviflora* was significantly reduced during the last four months (Table 5.1). Leaf formation and leaf fall varied greatly among both species where both leaf formation and leaf fall were less affected in *A. marina* under tidal treatments (Table 5.1). The number of new leaves produced in *A. marina* was significantly decreased in the deep treatment during the last month and leaf fall was not influenced by the tidal treatment (Table 5.1). However, deep treatment reduced leaf formation and induced leaf fall in *B. parviflora* during the last four months (Table 5.1). The two-way interaction between tidal treatment and time indicated significant interactive effects on height and stem diameter of *A. marina* but not on leaf formation and leaf fall

(Table 5.2). In *B. parviflora* a significant two-way interaction between tidal treatment and time was observed in height, stem diameter and leaf fall (Table 5.3).

Table 5.1. Statistical significance of the growth responses of *A. marina* and *B. parviflora* in tidal treatments (n = 40 per species)

Growth Parameter	Species	Treatment	Time (Months)						
			0	1	2	3	4	5	6
Height (cm)	*A. marina*	Deep	39.3 ± 2.6 [a]	42.1 ± 2.7 [a]	43.7 ± 2.7 [a]	45.0 ± 2.6 [a]	47.2 ± 2.5 [a]	48.8 ± 2.7 [a]	50.5 ± 2.8 [a]
		Shallow	40.4 ± 2.7 [a]	41.2 ± 2.7 [a]	42.4 ± 2.6 [b]	43.5 ± 2.7 [b]	44.7 ± 2.8 [b]	45.9 ± 3.0 [b]	47.0 ± 2.9 [b]
	B. parviflora	Deep	19.3 ± 3.5 [a]	20.4 ± 3.5 [a]	21.2 ± 3.5 [a]	22.2 ± 3.6 [a]	22.9 ± 3.6 [a]	24.0 ± 3.6 [a]	25.3 ± 3.4 [a]
		Shallow	19.6 ± 3.6 [a]	19.8 ± 3.5 [b]	20.6 ± 3.4 [b]	21.2 ± 3.4 [b]	21.6 ± 3.4 [b]	22.2 ± 3.5 [a]	23.0 ± 3.4 [a]
Stem Diameter (mm)	*A. marina*	Deep	6.11 ± 0.62 [a]	6.23 ± 0.63 [a]	6.31 ± 0.64 [a]	6.43 ± 0.64 [a]	6.56 ± 0.69 [a]	6.70 ± 0.70 [a]	6.79 ± 0.69 [a]
		Shallow	6.00 ± 0.63 [a]	6.17 ± 0.63 [a]	6.41 ± 0.63 [a]	6.65 ± 0.64 [a]	6.98 ± 0.73 [a]	7.24 ± 0.73 [b]	7.50 ± 0.72 [b]
	B. parviflora	Deep	4.61 ± 0.43 [a]	4.71 ± 0.43 [a]	4.84 ± 0.44 [a]	4.96 ± 0.43 [a]	5.21 ± 0.30 [a]	5.38 ± 0.31 [a]	5.56 ± 0.32 [a]
		Shallow	4.54 ± 0.48 [a]	4.76 ± 0.49 [a]	4.88 ± 0.49 [a]	5.29 ± 0.52 [b]	5.59 ± 0.37 [b]	5.78 ± 0.39 [b]	6.01 ± 0.39 [b]
Number of new leaves	*A. marina*	Deep	12.7 ± 3.2 [a]	13.6 ± 2.9 [a]	15.0 ± 2.8 [a]	16.6 ± 5.6 [a]	17.7 ± 2.5 [a]	19.2 ± 2.5 [a]	20.2 ± 2.3 [a]
		Shallow	13.3 ± 3.2 [a]	14.6 ± 3.1 [a]	16.3 ± 3.1 [a]	17.7 ± 3.0 [a]	19.1 ± 3.5 [a]	20.6 ± 3.5 [a]	22.3 ± 3.3 [b]
	B. parviflora	Deep	8.7 ± 2.1 [a]	10.0 ± 2.1 [a]	11.3 ± 2.3 [a]	12.7 ± 2.0 [a]	13.4 ± 1.8 [a]	14.8 ± 1.9 [a]	15.6 ± 2.2 [a]
		Shallow	8.6 ± 2.1 [a]	10.3 ± 2.1 [a]	12.1 ± 2.2 [a]	13.9 ± 2.2 [b]	16.0 ± 2.7 [b]	17.8 ± 2.7 [b]	19.1 ± 2.7 [b]
Leaf fall	*A. marina*	Deep	1.9 ± 1.3 [a]	2.6 ± 1.3 [a]	4.2 ± 1.4 [a]	5.8 ± 1.7 [a]	7.4 ± 1.8 [a]	9.5 ± 1.7 [a]	11.4 ± 1.9 [a]
		Shallow	1.8 ± 1.3 [a]	2.5 ± 1.3 [a]	4.3 ± 1.6 [a]	5.9 ± 1.9 [a]	7.8 ± 2.3 [a]	9.8 ± 2.4 [a]	11.8 ± 2.1 [a]
	B. parviflora	Deep	2.4 ± 1.0 [a]	3.0 ± 0.9 [a]	4.0 ± 1.0 [a]	5.0 ± 1.3 [a]	6.8 ± 0.8 [a]	8.2 ± 1.3 [a]	9.2 ± 0.8 [a]
		Shallow	2.3 ± 0.9 [a]	3.1 ± 0.7 [a]	3.8 ± 1.0 [a]	4.3 ± 1.1 [b]	5.0 ± 0.8 [b]	5.1 ± 0.8 [b]	5.3 ± 0.7 [b]

Mean and standard deviation, means in the same column with different letters in the same column are significantly different at α 0.05

Table 5.2. Repeated measures of ANOVA results for the two-way interaction between tidal treatment and time on growth parameters of *A. marina*

Parameter (*Source of Variation*)	***df***	**MS**	***F***	***p***
Height				
Treatment	1	30.104	179.4	<0.0001
Time	5	1.617	3.3	0.008
Treatment x Time	5	7.529	7.5	<0.0001
Diameter				
Treatment	1	0.793	201.5	<0.0001
Time	5	0.021	7.2	<0.0001
Treatment x Time	5	0.022	7.8	<0.0001
Number of Leaves				
Treatment	1	4.817	6.2	0.023
Time	5	2.27	3.0	0.014
Treatment x Time	5	1.177	1.3	0.261
Leaf Fall				
Treatment	1	0.004	0.006	0.938
Time	5	9.034	5.8	<0.0001
Treatment x Time	5	0.254	0.177	0.971

Table 5.3. Repeated measures of ANOVA results for the two-way interaction between tidal treatment and time on growth parameters of *B. parviflora*

Parameter (*Source of Variation*)	***df***	**MS**	***F***	***p***
Height				
Treatment	1	15.251	159.9	<0.0001
Time	5	0.549	2.6	0.032
Treatment x Time	5	0.839	4.3	0.001
Diameter				
Treatment	1	0.451	167.9	<0.0001
Time	5	0.114	30.6	<0.0001
Treatment x Time	5	0.134	39.1	<0.0001
Number of Leaves				
Treatment	1	12.150	26.1	<0.0001
Time	5	2.177	2.8	0.02
Treatment x Time	5	0.310	0.464	0.802
Leaf Fall				
Treatment	1	16.538	97.8	<0.0001
Time	5	0.544	1.18	0.323
Treatment x Time	5	2.158	7.3	<0.0001

5.3.2 Biomass Allocation and RGR

Biomass and biomass allocation between deep and shallow treatments varied among *A. marina* and *B. parviflora*. The total biomass and biomass allocations in roots and shoots of *A. marina* were not significantly affected by the tidal treatments during the first three months (Table 5.4). However, at the end of the study period, a significant decrease in the root biomass and a significant increase in shoot biomass of *A. marina* were observed (Table 5.4). Similarly, a significant decrease in R/S ratio was observed. The RGR of *A. marina* remained unaffected by the tidal treatments throughout the study period (Table 5.4).

In contrast to *A. marina*, the biomass and biomass allocations of *B. parviflora* varied significantly between deep and shallow treatments. In the deep treatment, the root, shoot and total biomasses of *B. parviflora* were significantly reduced throughout the study period (Table 5.4). The R/S ratio remained unaffected by the tidal treatments during the first three months but a significant reduction in R/S ratio was observed in the deep treatment at the end of the study period (Table 5.4). The RGR of *B. parviflora* in the deep treatment was significantly reduced throughout the study period (Table 5.4).

Table 5.4. Biomass allocations of *A. marina* and *B. parviflora* seedlings subjected to two tidal treatments in the greenhouse (n = 36)

Biomass parameter	Species	Treatment	Time (Months)	
			3	6
Root (mg)	***A. marina***	Shallow	0.65 ± 0.08 [a]	0.82 ± 0.09 [a]
		Deep	0.66 ± 0.07 [a]	0.75 ± 0.10 [b]
	B. parviflora	Shallow	0.48 ± 0.06 [a]	0.59 ± 0.06 [a]
		Deep	0.42 ± 0.06 [b]	0.49 ± 0.06 [b]
Shoot (mg)	***A. marina***	Shallow	1.05 ± 0.10 [a]	1.12 ± 0.10 [a]
		Deep	1.03 ± 0.08 [a]	1.20 ± 0.10 [b]
	B. parviflora	Shallow	0.80 ± 0.08 [a]	0.91 ± 0.09 [a]
		Deep	0.73 ± 0.07 [b]	0.81 ± 0.08 [b]
Total (mg)	***A. marina***	Shallow	1.70 ± 0.17 [a]	1.94 ± 0.19 [a]
		Deep	1.69 ± 0.15 [a]	1.95 ± 0.19 [a]
	B. parviflora	Shallow	1.28 ± 0.13 [a]	1.49 ± 0.13 [a]
		Deep	1.15 ± 0.12 [b]	1.31 ± 0.13 [b]
R/S	***A. marina***	Shallow	0.62 ± 0.06 [a]	0.73 ± 0.03 [a]
		Deep	0.64 ± 0.03 [a]	0.63 ± 0.04 [b]
	B. parviflora	Shallow	0.61 ± 0.05 [a]	0.65 ± 0.04 [a]
		Deep	0.58 ± 0.05 [a]	0.61 ± 0.05 [b]
RGR	***A. marina***	Shallow	0.62 ± 0.06 [a]	0.73 ± 0.03 [a]
		Deep	0.64 ± 0.03 [a]	0.63 ± 0.04 [a]
	B. parviflora	Shallow	0.61 ± 0.05 [a]	0.65 ± 0.04 [a]
		Deep	0.58 ± 0.05 [b]	0.61 ± 0.05 [b]

Mean and standard deviation, means in the same column with different letters in the same column are significantly different at α 0.05

5.3.3 Soil Physical Parameters

The initial pH of *A. marina* and *B. parviflora* were 6.77 ± 0.15 and 6.75 ± 0.22 respectively. There was no significant difference in the initial soil pH of both species ($t_{2(78)} = 0.64$, $p = 0.526$). The variations in the soil physical characteristics at the end of six months revealed interesting results in terms of treatments and species (Figure 5.1) The results of the two-

way ANOVA indicated significant differences in the pH and Eh of the tidal treatments as well as between both species (Table 5.5). In the deep treatment of *A. marina*, the decrease in pH and Eh compared to that of shallow treatment was 0.93 and -16 respectively. In *B. parviflora* the decrease in pH and Eh of the deep treatment was 1.39 and -30 respectively. Soil physical characteristics were also affected by the species where a significant difference in soil pH and Eh was observed between *A. marina* and *B. parviflora* (Table 5.5). Likewise, a significant two-way interaction was observed in the soil pH and Eh between the species and treatments (Table 5.5).

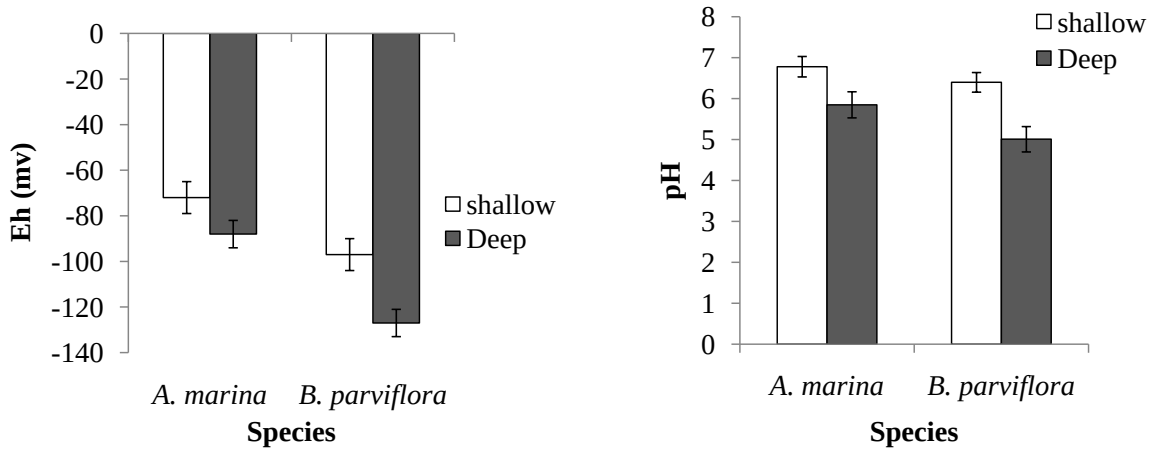


Figure 5.1. Variations in the soil pH and Eh of *A. marina* and *B. parviflora* in tidal treatments (n = 40)

The correlation analyses of the soil physical parameters and the RGR and R/S ratio of *A. marina* (Table 5.6) revealed no significant relationships between RGR and the soil physical parameters. However, soil pH and Eh had a significant positive effect on the R/S ratio. In contrast, RGR of *B. parviflora* showed significant positive correlations between both pH

and Eh. The R/S ratio had a significant positive relationship between soil pH but was not affected by the variation in the soil Eh.

Table 5.5. Two-way ANOVA results of the soil physical characteristics between treatments and species

Parameter (*Source of Variation*)	***df***	**MS**	***F***	***p***
pH				
Species	1	7.405	89.5	<0.0001
Treatment	1	26.842	324.5	<0.0001
Species x Treatment	1	1.017	12.3	0.001
Eh(mv)				
Species	1	20512.013	114	<0.0001
Treatment	1	10465.312	58.2	<0.0001
Species x Treatment	1	812.813	4.5	0.037

Table 5.6. Correlation analyses between soil physical characteristics and RGR and R/S ratio of *A. marina* and *B. parviflora*

			RGR	**R/S**
A. marina	**pH**	Correlation coefficient	-0.055	0.615
		Significance (*p*)	0.734	<0.0001
		N	40	40
	Eh (mv)	Correlation coefficient	0.177	0.406
		Significance (*p*)	0.275	0.009
		N	40	40
B. parviflora	**pH**	Correlation coefficient	0.807	0.365
		Significance (*p*)	<0.0001	0.02
		N	40	40
	Eh (mv)	Correlation coefficient	0.664	0.092
		Significance (*p*)	<0.0001	0.571
		N	40	40

5.4 DISCUSSION

5.4.1 Seedling Growth

The growth responses of *A. marina* and *B. parviflora* varied between tidal treatments. As observed in several other species subjected to tidal flooding, both *A. marina* and *B. parviflora* has induced stem height increments in the deep treatment. Similarly, variations in the stem height were affected by the interaction of both tidal treatment and time. Stem height increment of *A. marina* was constantly higher throughout the study period in the deep treatment while no significant difference in stem height was observed in *B. parviflora* towards the end of the study (Table 5.1). At the end of the study period, *A. marina* seedlings grew above the water level of the deep treatment and were in contact with the aerial environment. Stem height increments under tidal flooding has been observed in other mangrove species such as *Bruguiera gymnorrhiza* and *Kandelia candel* (Ye et al., 2003), *Laguncularia racemosa* (Delgado et al., 2001) and *Rhizophora mangle* (Ellison and Farnsworth, 1997).

One of the mechanisms underlying stem elongation of plants under submergence is ethylene-induced stem elongation (Jackson, 2008). It is one of the well inferred hormone-mediated stress adaptations by plants that help to restore contact with the aerial environment by decreasing the duration of tidal inundations (Jackson and Colmer, 2005). The ability to escape above the water-surface is generally accepted as a characteristic feature of those species that are well adapted to waterlogging (Jackson, 2008). Thus, constant stem height increments of *A. marina* in the deep treatment suggest better tolerance to prolonged waterlogging than *B. parviflora*.

In contrast to stem height, the stem diameter of *A. marina* and *B. parviflora* was decreased in the deep treatment (Table 5.1). A significant decrease in the diameter at the first stem node of *A. marina* was observed during the last two months while a significant decrease was observed in *B. parviflora* during the last four months. Different responses in stem diameter increment have been reported in different species under tidal flooding. For instance, tidal flooding decreased stem diameter of *B. gymnorrhiza* under tidal flooding while stem diameter increment of *K. candel* was insensitive to tidal flooding. Stem diameter increment observed in this study suggest that *A. marina* is less sensitive to tidal flooding than *B. parviflora*.

The responses of leaf formation and leaf fall; varied extensively between both species (Table 5.1). Leaf formation in *A. marina* was not affected by the tidal treatment until the last month and leaf fall was insensitive to tidal flooding. Likewise, the interactive effect of tidal treatments and time was insignificant on leaf formation and leaf fall of *A. marina*. In contrast, a significant decrease in leaf formation and a significant increase in leaf fall were observed in *B. parviflora* during the last four months and both leaf formation and leaf fall were significantly affected by the interaction of tidal treatment and time. These results suggest that in terms of leaf formation and conservation, *A. marina* is less sensitive to tidal flooding than *B. parviflora*. Similar results have been reported on *A. marina*. In a comparative study of four mangrove species in a diurnal tidal zone, where leaf numbers, leaf area and leaf conservation of *A. marina* increased in the lower tidal flood elevations (He et al., 2007).

One possible mechanism attributed to the variations in the growth responses of *A. marina* and *B. parviflora* under tidal flooding is hormone-mediated growth responses to abiotic

stresses. The main hormone that imparts tolerance to environmental stresses in plants is abscisic acid (ABA) and the endogenous concentrations of which is influenced by the environmental conditions (Kurepin et al., 2008; Wasilewska et al., 2008). ABA is the key hormone that regulate leaf senescence and increased levels of ABA have been reported in the mature leaves of *K. candel* seedlings under prolonged tidal inundation (Luzhen et al., 2005). Furthermore, increased levels of ABA inhibit submergence-induced stem elongation (Voesenek et al., 2004). Thus, decreased stem height and increased leaf fall in *B. parviflora* towards the end of the study could result from the increased levels of ABA in response to tidal flooding. In contrast in the deep treatment continuous height increment of *A. marina* was observed and leaf conservation was insensitive to tidal flooding. The variations in the growth responses of *A. marina* and *B. parviflora*, therefore, suggest *B. parviflora* is more sensitive to tidal flooding while *A. marina* is better adapted to cope with the stresses associated with prolonged tidal inundation. The differential ability to with stand could explain the huge difference in growth of these two species, where growth of *A. marina* is much rapid than that of *B. parviflora*.

5.4.2 Biomass Allocation and RGR

The total biomass and RGR of *A. marina* remained unaffected by the tidal treatments but the total biomass and RGR of *B. parviflora* was significantly decreased in the deep treatment throughout the study period (Table 5.4). Increased biomass of *A. marina* was observed in the lower tidal flat elevations and the biomass and RGR of *A. marina* was less variable between tidal treatments (He et al., 2007). As observed in *B. parviflora*, decreased biomass under tidal flooding has been reported in *A. germinans* (Pezeshki et al., 1997) and

R. mangle (Pezeshki et al., 1990b). In a comparative study between *K. candel* and *B. gymnorrhiza*, decreased biomass and RGR was observed in *B. gynmorrhiza* while simulated sea level rise did not affect these parameters of *K. candel* (Ye et al., 2003). Thus, different mangrove species exhibit different responses to tidal flooding.

The major constraint faced by the growing seedlings under prolonged tidal flooding is the restriction of gaseous exchange between the soil surface and the atmosphere (Pezeshki, 2001). Thus, many plants exhibit mechanisms to sustain minimal oxygen loss through respiration to ensure enhanced survival rates. Similarly, the ability to withstand tidal flooding in mangrove plants may be attained through reduced photosynthesis to levels that just sustain maintenance. This decrease in photosynthesis is often attributed to increased levels of ABA that induce stomatal closure causing limited gas exchange and metabolic inhibition (Pezeshki, 1993). Thus, decreased total biomass and RGR of *B. parviflora* could result from combined effects of decreased assimilation and increased leaf fall under tidal flooding. The variations in the RGR of *A. marina* and *B. parviflora* suggest adverse effects of tidal flooding on *B. parviflora* but less sensitivity to tidal flooding in *A. marina* which further suggests that *A. marin*a is better adapted to cope with rising sea levels.

The biomass allocations to above and below ground regions varied between both species. In the deep treatment, the root biomass decreased while shoot biomass increased in *A. marina*. In contrast, both root and shoot biomass of *B. parviflora* decreased in the deep treatment. These results indicate significant variations in the responses of *A. marina* and *B. parviflora* under tidal flooding. However, both species had decreased R/S ratio in the deep treatment. The decreased biomass allocation to the roots relative to the shoots was observed in *K. candel* and *B. gymnorrhiza* under tidal flooding (Ye et al., 2004). The decreased R/S ratio

under tidal flooding could decrease the oxygen demand by the root tissue, facilitate oxygen availability in the growing root tip by shortening the diffusion path, and reduce the amounts of oxygen needed for external rhizosphere oxidation (Ye et al., 2004). A greater decrease in the R/S ratio was observed in *A. marina* than in *B. parviflora*, indicating that both species exhibit certain degree of tolerance to periods of tidal inundations but may differ in their ability to withstand prolonged tidal inundation. Thus, based on these results it is indicative that *A. marina* is better adapted to survive under prolonged tidal inundations.

5.4.3 Soil Physical Parameters

The soil physical characteristics varied significantly between tidal treatments (Table 5.5; Figure 5.1). Reduced soil ph and Eh in the deep treatment indicated decreased oxygen concentrations in the soil. Under tidal flooding the limited supply of oxygen in the soil is readily used up by roots, microorganisms and soil reductants causing extensive soil reduction (Ponnamperuma, 1984). Decreased soil reduction results in a series of soil chemical changes that lead to further reductions in the soil causing several changes in the soil physicochemical characteristics (Gambrell and Patrick, 1978). Mangrove plants respond to changes in the soil physicochemical characteristics and the oxygen demand in the root medium regulate wetland plant functioning (Delaune and Pezeshki, 1991). These responses often lead to a variety of stress symptoms but extensive variations in these responses may be observed in different species (Drew, 1990; Kozlowski, 1984a; Vartapetian and Jackson, 1997).

The soil physical characteristics varied significantly between both species (Table 5.5). Greater decreases were observed in the soil physical parameters in the deep treatment of *B.*

parviflora than that of *A. marina*. Similarly, correlation analyses between soil physical parameters and RGR and R/S ratio indicated differences in these parameters between both species (Table 5.6). Positive correlations between R/S ratio and the soil pH and Eh suggest that with *A. marina* invested less as the soil pH and Eh decreased which further suggest that reduced R/S ratio in the deep treatment is a substantial adaptation exhibited in *A. marina* to prolonged tidal inundation. Furthermore, ability to decrease oxygen demand under reduced soil conditions in the deep treatment could explain the similar RGR observed in both tidal treatments of *A. marina*. Unlike *A. marina*, there was no significant relationship between R/S ratio and soil Eh of *B. parviflora*. Likewise, as the soil pH and Eh decreased the RGR decreased inducing stunted growth of *B. parviflora* in the deep treatment. Similar results have been reported where increased soil acidity under simulated sea level rise caused stunted growth of *R. mangle* seedlings (Ellison and Farnsworth, 1997). Thus, variations in soil physical characteristics affected growth of *B. parviflora* more than that of *A. marina*.

Decreased oxygen concentrations in the soil may lead to excessive oxygen loss from the roots into the rhizosphere, a process known as radial oxygen loss (ROL) (Armstrong et al., 1992). Mangrove plants that are well adapted to withstand extensive soil reduction often develop a barrier to ROL in subapical root zone to prevent excessive loss of diffusing oxygen to the anaerobic soils (Pi et al., 2009). Reduced oxygen loss increases diffusion of oxygen towards the root tips (Visser et al., 2000). However, the performance of this barrier is species specific as the efficiency of this barrier is related to the hypodermal structure, suberin composition and distribution within exodermal cell walls (Soukup et al., 2007), which is determined by the root anatomical features such as exodermal and hypodermal structures that vary among different mangrove plants (Pi et al., 2009; Youssef and Saenger,

1996). Thus, the difference in the efficiency of the ROL barrier of *A. marina* and *B. parviflora* could lead to decreased soil redox potentials in *B. parviflora* under prolonged inundation. In a comparative study on root anatomy and spatial pattern of radial oxygen loss of eight mangrove species, Pi et al., (2009) reported highest tolerance to waterlogging in *A. marina*. In the same study, *A. marina* was found to have a weak ROL barrier but had largest areas of aerenchyma air spaces that significantly enhanced efficient gaseous exchange under tidal flooding.

5.5 CONCLUSION

Growth and biomass partitioning between *A. marina* and *B. parviflora* varied significantly between tidal treatments suggesting that these two species responded differently under tidal inundation. Less variation in growth and biomass allocations of *A. marina* under tidal treatments suggest that *A. marina* is better adapted to cope with prolonged tidal inundation which supports the initial hypothesis that these two species vary in their tolerance to tidal inundation. However, increased tidal flooding had adverse effects on *B. parviflora* which resulted in stunted growth and reduced biomass. Similarly, leaf formation and leaf fall of *B. parviflora* were significantly affected in deep treatment. The variation in the ability to withstand prolonged tidal inundation could explain the intertidal distribution of these two species where *A. marina* predominate lower intertidal zones and are often subjected to increased tidal inundation whereas *B. parviflora* is more restricted to landward margins of the mangrove forests. Similarly, soil physical characteristics varied significantly between both species. Soil reduction was more intense in the deep treatment of *B. parviflora* suggesting that aeration of immediate rhizosphere in this species was less compared to *A.*

marina. The variations in soil physical characteristics further suggest that *A. marina* is better adapted to survive under rising sea levels. Furthermore, species that exhibit rapid growth in terms of increased stem elongation bear advantages over those species with slow growth forms when exposed to tidal inundation.

CHAPTER 6 - PHYSIOLOGICAL RESPONSES OF *AVICENNIA MARINA* VAR. *ACUTISSIMA* AND *BRUGUIERA PARVIFLORA* UNDER SIMULATED SEA LEVEL RISE

6.1 INTRODUCTION

Mangroves, like other ecosystems, are threatened by the current climate change. Two major components of the climate change that are likely to affect mangrove communities are increased atmospheric carbon dioxide (CO_2) and rising sea levels (Krauss et al., 2008). Increased atmospheric CO_2 could enhance growth rates via increased net assimilation rates and increased leaf area ratio and via increased water use efficiency (Ball et al., 1997). Likewise increased atmospheric CO_2 concentrations determine stomatal regulation of water loss (Morison, 1998). However, the extend to which individual species respond to increased CO_2 depends on other growth limiting factors such as nutrient availability and presence of stress factors such as salinity and flooding (Ball et al., 1997; McKee and Rooth, 2008).

Mangroves are exposed to various levels of tidal inundations depending on the geomorphological characteristics of the shoreline (Woodroffe and Grindrod, 1991). Sea level rise is likely to prolong tidal inundation and cause intense soil anoxia (Pezeshki, 2001). Under experimental cultures, soil flooding can reduce oxygen concentrations of the soil by as much as 28% after 6 hours of flooding and as much as 72% after 20 hours (Skelton and Allaway, 1996). Intense soil anoxia trigger various physiological responses in mangroves (Pezeshki, 2001). Soil flooding reduced light saturation levels and photosynthesis of *Kandelia candel* seedlings (Chen et al., 2005), decreased stomatal conductance and leaf xylem water potential of *A. marina* (Sayed, 1995), while tidal

flooding enhanced biomass attributed of *Xylocarpus granatum* (Allen et al., 2003). Thus mangrove plants exhibit variations in the physiological responses to tidal inundations.

The combined effects of increased and sea level rise on mangroves are poorly understood. The aim of the current study is to evaluate the physiological response of *A. marina* var. *acutissima* and *Bruguiera parviflora* under simulated sea level rise and to determine the carbon sequestration of these species when subjected to different tidal treatments. The previous study on the growth of *A. marina* var. *acutissima* and *B. parviflora* indicated differential growth responses under simulated sea level rise where *B. parviflora* had reduced growth (Chapter 5). In light of the results obtained in the previous study, it is hypothesized that; firstly, physiological responses of *A. marina* var. *acutissima* and *B. parviflora* will vary between tidal treatments; secondly, carbon sequestration of both species will be affected by the tidal treatments; and thirdly, the effects of tidal flooding will affect *A. marina* to a lesser extent than that of *B. parviflora*.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Design and Culture Conditions

The experimental setup of simulated sea level rise in the greenhouse was the same as that described in section 5.2.1 with two tidal treatments; deep and shallow. The seedlings were cultured according to the methods stated in section 5.2.2.

6.2.2 Physiology and Carbon Sequestration

The physiological data collection was carried out at the end of sixth month after subjecting the seedlings to the treatments. Six seedlings from each treatment of each species were

randomly selected to study physiological variations between tidal treatments. A portable photosynthesis system (PPSystem, CIRAS-1, USA) was used to obtain carbon sequestration data on *A. marina* var. *acutissima* and *B. parviflora* seedlings according to the methods described in section 4.2.2.

The assimilation-carbon dioxide response curves were fit for individual plants by a non-linear regression to hyperbolic equation. Maximum photosynthetic rates (A_{max}), photosynthetic rates at ambience (A_{400}), and CO_2 compensation point were determined according to the methods described in section 4.2.2. The ratio A_{400}/A_{max} was obtained to determine the deviation of the current photosynthetic rate from the maximum value that the species can reach under elevated atmospheric CO_2. The lower the ratio A_{400}/A_{max}, the higher the carbon sink potential of that species; which indicates that the species can increase its photosynthetic rate with further increasing atmospheric CO_2 in the future. Chlorophyll conductance of the seedlings was recorded according to the methods described in section 4.2.2.

Comparisons of the physiological and ecological parameters were made in order to determine the relationships between A_{400} and relative growth rates (RGR) and root to shoot ratio (data from 5.3.2). Similarly, relationships of A_{400} and A_{max} between soil physical parameters (data from 5.3.3) were evaluated in order to determine the relationships between these physiological parameters and soil physical parameters.

6.2.3 Data Analysis

Analysis of Variance (ANOVA) was performed to determine any variations in the physiological parameters and chlorophyll conductance between two predictor variables; treatment and species. Likewise ANOVA was used to determine the differences in the rate of assimilation between treatment, species and carbon dioxide concentrations. Post-hoc statistical groupings were observed with a Turkey's test at $\alpha = 0.05$. All the analyses were performed with SPSS version 16.0 software. Correlation analyses were performed to determine the relationship between physiological and both ecological parameters and soil physical parameters.

6.3 RESULTS

Carbon sequestration of *A. marina* and *B. parviflora* indicated interesting results (Table 6.1; Figure 6.1). The variations in the tidal treatment affected physiological parameters of *A. marina* and *B. parviflora*. Prolonged tidal inundation in the deep decreased A_{400} of *B. parviflora* but no significant difference in A_{400} was observed in *A. marina*. In contrast, carbon dioxide compensation point (Γ_{CO_2}) was not affected by the tidal treatment but varied significantly between *A. marina* and *B. parviflora*. In both species, A_{max} was significantly lower in the deep treatment and A_{max} of *B. parviflor*a was significantly lower than that of *A. marina*. Significant two-way interactions between tidal treatment and species were observed. The ratio between A_{400} and A_{max} of both species was lower in the shallow treatment than in the deep treatment. In *A. marina*, the difference in A_{400}/A_{max} was less between tidal treatments compared to *B. parviflora*.

Chlorophyll conductance of *A. marina* and *B. parviflora* was not affected by the tidal treatments (Table 6.1; Figure 6.2). However, chlorophyll conductance of *B. parviflora* was significantly higher compared to *A. marina*.

Table 6.1. Two-way ANOVA results of the physiological parameters between treatments and species

Parameter (*Source of Variation*)	***df***	**MS**	***F***	***p***
$A_{(max)}$				
Species	1	578.104	37.8	<0.0001
Treatment	1	1382.443	90.5	<0.0001
Species x Treatment	1	82.993	5.4	0.030
$A_{(400)}$				
Species	1	366.914	11.6	0.003
Treatment	1	693.805	21.9	<0.0001
Species x Treatment	1	52.570	1.7	0.212
Γ_{CO2}				
Species	1	95.600	5.7	0.027
Treatment	1	29.560	1.7	0.202
Species x Treatment	1	0.570	0.03	0.856
$A_{(400)}/A_{(max)}$				
Species	1	0.005	0.5	0.476
Treatment	1	0.023	2.5	0.129
Species x Treatment	1	0.002	0.3	0.613
Chlorophyll conductance				
Species	1	5185.81	164.5	<0.0001
Treatment	1	73.153	2.3	0.132
Species x Treatment	1	8.646	0.274	0.602

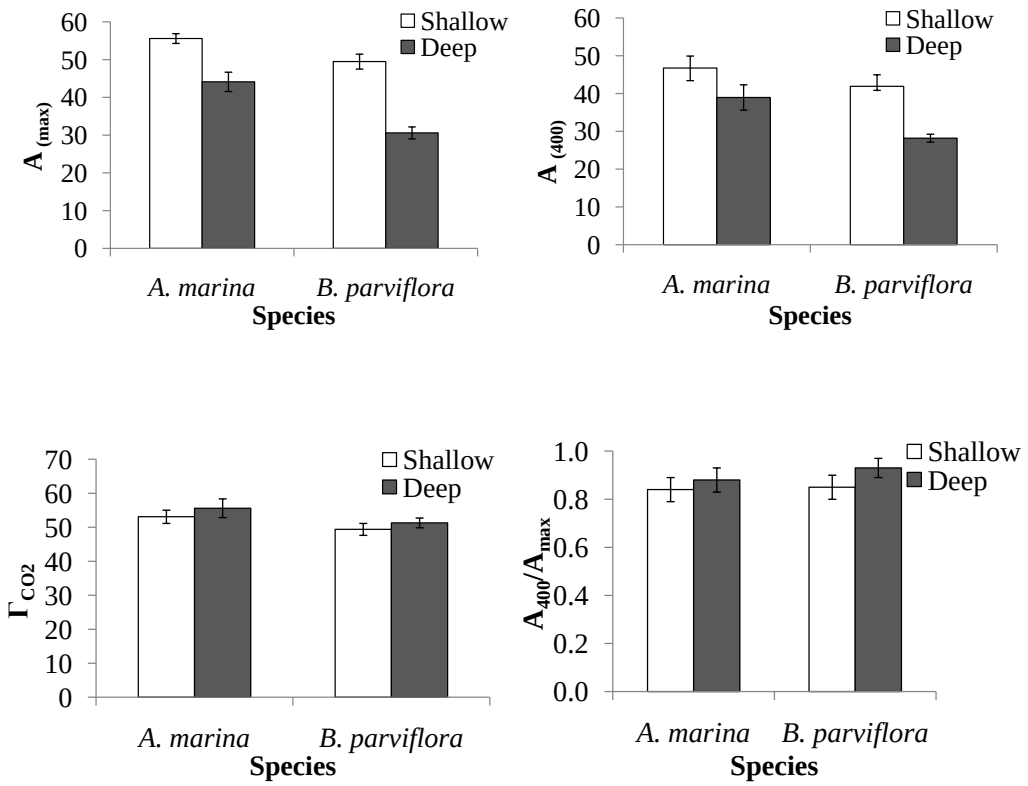


Figure 6.1. Variations in A_{max}, A_{400}, Γ_{CO_2} and A_{400}/A_{max} of *A. marina* and *B. parviflora* in tidal treatment

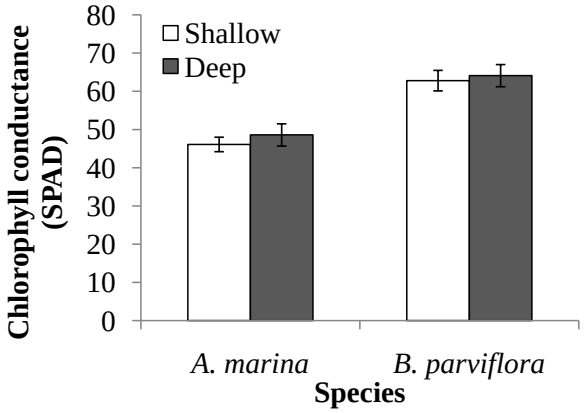


Figure 6.2. Chlorophyll conductance of *A. marina* and *B. parviflora* in tidal treatments

In *A. marina*, increased assimilation was observed with increased CO_2 in both tidal treatments, but this increase was much rapid in the shallow treatment compared to the deep treatment (Figure 6.3). Assimilation rate of *B. parviflora* increased with increasing CO_2 in the shallow treatment. Increased CO_2 did not increase assimilation in the deep treatment and CO_2 saturation was observed below ambience. The assimilation rates varied significantly between species, treatments and CO_2 concentrations. However, the three-way interaction between species, treatment and CO_2 concentration was not significant (Table 6.2).

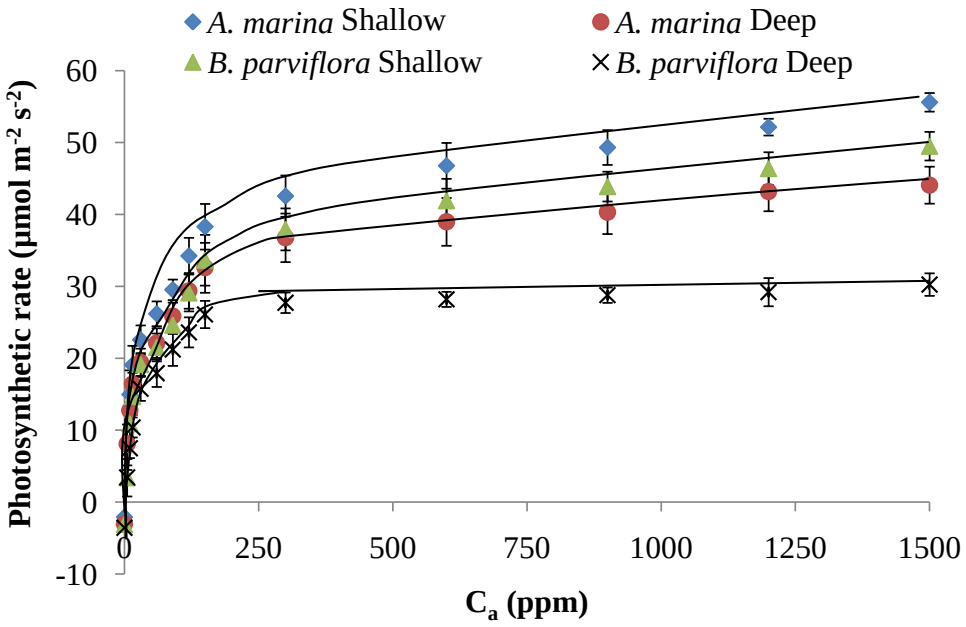


Figure 6.3. CO_2 response curves for *A. marina* and *B. parviflora* in tidal treatments at PAR 1800 µmol m^{-2} s^{-1}

Table 6.2. Three-way ANOVA results of assimilation between species, treatments and CO_2 concentrations

Parameter (*Source of Variation*)	***df***	**MS**	***F***	***p***
Assimilation (µmol m^{-2} s^{-1})				
Species	1	2882.652	145.4	<0.0001
Treatment	1	3364.522	169.7	<0.0001
CO_2	13	5159.369	262.1	<0.0001
Species x Treatment	1	145.676	7.3	0.007
Species x CO_2	13	38.18	1.9	0.027
Treatment x CO_2	13	138.807	7	<0.0001
Species x Treatment x CO_2	13	15.054	0.759	0.703

The correlation analyses of the physiological parameters and ecological parameters indicated significant positive correlations between A_{400} and RGR (Figure 6.4 (a)). However, there was no significant relationship between A_{400} and R.S ratio (Figure 6.4 (b)). Soil physical characteristics had positive effects on A_{max} and A_{400} (Figure 6.5). Soil pH had significant positive effects on both A_{max} and A_{400} whereas soil Eh had a significant positive effect on A_{max}.

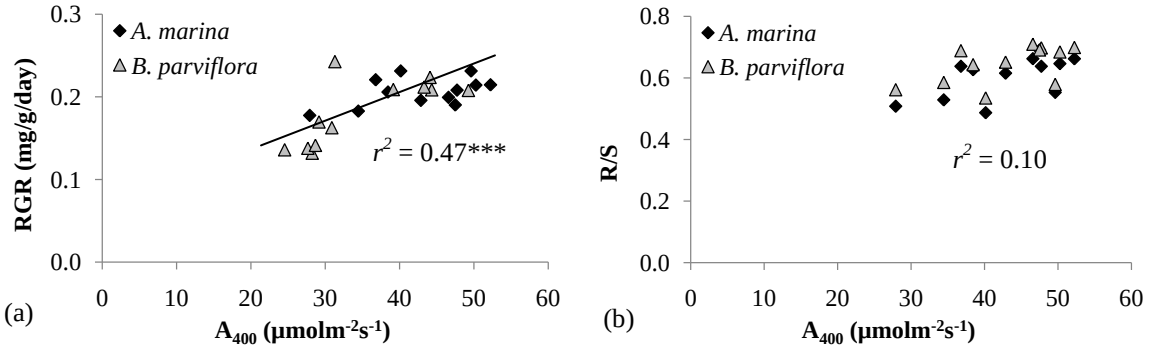


Figure 6.4. Relationship between (a) A_{400} and RGR (n – 24); and (b) A_{400} and R/S ratio (n = 24). Significance is denoted by *** < 0.001

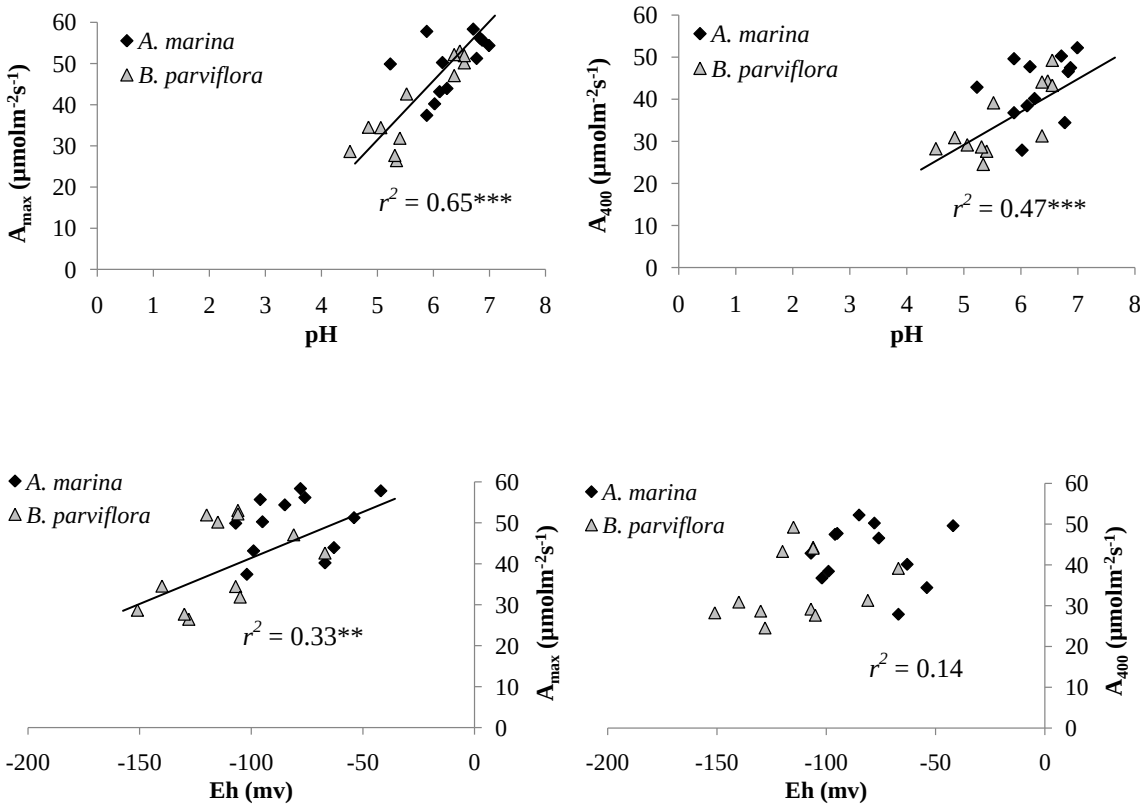


Figure 6.5. Relationship of A_{max} and A_{400} between soil physical parameters (n = 24). Significance is denoted by ** < 0.01, *** < 0.001

6.4 DISCUSSION

Prolonged tidal inundation decreased both A_{max} and A_{400} of *B. parviflora* while tidal flooding only affected A_{max} of *A. marina* indicating that assimilation rate of *A. marina* was unaffected at ambient CO_2 concentration by the tidal flooding (Table 6.1; Figure 6.1). These results correspond well with the growth responses observed in these two species in the previous study where tidal inundation caused adverse effects on growth of *B. parviflora* but not on *A. marina*. Similarly, A_{max} and A_{400} were much higher in *A. marina* at a given treatment than *B. parviflora*, which explains, to some extent, the variations in the growth

forms of these two species. The lower A_{400}/A_{max} in both species in the shallow treatment suggests that these two species can withstand increased CO_2 concentrations under moderate tidal flooding. In *A. marina*, the ratio of A_{400}/A_{max} was less variable between tidal treatments indicating that this species can withstand CO_2 concentrations even under prolonged tidal inundation. In contrast, A_{400}/A_{max} of *B. parviflora* was significantly lower in the shallow treatment indicating that this species can only maximize photosynthesis with increasing CO_2 concentrations under moderate tidal inundation. Reduced assimilation under tidal flooding have been reported in *K. candel* (Chen et al., 2005) and *R. mangle* (Ellison and Farnsworth, 1997). Similarly, short term flooding decreased leaf-level carbon assimilation in *A. germinans*, *L. racemosa* and *R. mangle* while long term flooding had slight effects on the leaf gas exchange parameters of these species (Krauss et al., 2006b).

Tidal treatments did not affect chlorophyll conductance of both *A. marina* and *B. parviflora* (Table 6.1; Figure 6.2). However, chlorophyll conductance of *B. parviflora* was much higher compared to *A. marina* in both tidal treatments. Reduced chlorophyll conductance have been observed in some wetland plants grown in flooded soils (McKevlin et al., 1995; Ye et al., 2004) while no significant differences were observed in others (Pezeshki et al., 1996). The increased chlorophyll conductance of *B. parviflora* in this study did not increase the assimilation rates.

Carbon sequestration of *A. marina* and *B. parviflora* was affected by the tidal treatment and significant differences were observed between both species (Table 6.2; Figure 6.3). Carbon sequestration of *B. parviflora* was substantially reduced in the deep treatment while the difference between tidal treatments was much less in *A. marina*. Decreased assimilation under prolonged tidal inundation is often associated with stomatal closure. Stomatal closure

resulting from changes in the guard cell turgor have been reported in mangroves (Naidoo et al., 1997). Decreased stomatal conductance under prolonged tidal inundation has been reported in *A. marina,* (Sayed, 1995), *B. gymnorrhiza* (Naidoo, 1983) and *K. candel* (Ye et al., 2004). Similarly, increased CO_2 induced stomatal closure to enhance photosynthetic water use efficiency (Urban, 2003). The results of carbon sequestration, therefore, suggest that *A. marina* is likely to benefit from increased atmospheric carbon dioxide even under prolonged tidal inundation. In contrast, the benefits of increased CO_2 are likely to be compensated for in *B. parviflora* by reduced growth in response to longer hydroperiods under sea level rise. In addition to stomatal closure, another potential mechanism that reduces photosynthesis under tidal flooding could be hormone-mediated physiological variations. For instance, the amount of ethylene has been associated with decreased photosynthesis resulting from lack of mesophyll photosynthetic capacity (Taylor and Gunderson, 1988). Alternatively, reduced photosynthesis could arise from altered enzymatic activity such as reduced rubisco activity induced by the decreased leaf water potential (Pezeshki, 1994).

The relationship between ecological and physiological parameters indicated significant positive effects of A_{400} on RGR (Figure 6.4). These results indicate that at CO_2 concentration of 400 ppm, both species are able to photosynthesis at rates that contribute to significant increases in RGR. Decreased soil pH and Eh under tidal flooding contribute major ecological and physiological constraints on the growing seedling (Unger et al., 2009). Both soil pH and Eh had significant positive effects on A_{max} indicating that higher A_{max} is attained under low acidic and reduced soil conditions (Figure 6.5). Soil pH also had a

significant positive effect on A_{400} suggesting that higher photosynthetic rates at CO_2 concentrations of 400 ppm are achieved in less acidic soil conditions.

6.5 CONCLUSION

The physiological responses of *A. marina* and *B. parviflora* under tidal treatments indicated that these two species responded differently. In *A. marina*, the only physiological parameter that varied between tidal treatments was A_{max} while in *B. parviflora* A_{max} and A_{400} was significantly reduced when exposed to tidal flooding. The difference in A_{max} and A_{400} was not limited by the chlorophyll conductance of both species as chlorophyll conductance was insensitive to tidal treatments. Hence the first hypothesis which states that physiological responses of *A. marina* var. *acutissima* and *B. parviflora* will vary between tidal treatments is accepted. Similarly, carbon sequestration of *A. marina* and *B. parviflora* was reduced in the deep treatment but the reduction was much prominent in *B. parviflora*. Thus, the second hypothesis which states that carbon sequestration of both species will be affected by the tidal treatments is accepted. Overall, *A. marina* showed fewer responses to tidal flooding than *B. parviflora*. Therefore the third hypothesis which states that tidal flooding will affect *A. marina* to a lesser extent than that of *B. parviflora* is accepted. These results indicate that *A. marina* is better adapted than *B. parviflora* for the projected climate change. Positive correlations were observed between some physiological and both ecological and soil physical parameters.

CHAPTER 7 - GENERAL CONCLUSION

7.1 INTRODUCTION

Rising sea levels associated with the current climate change pose great threats to mangrove ecosystems that occupy the interface between the sea and the land. Several studies on simulated sea level rise provide ample evidence on the differential abilities of mangrove species to tolerate prolonged tidal inundation. The aims of the current study were to evaluate the ecological and physiological responses of three dominant mangrove species; *Avicennia alba*, *A. marina* and *Bruguiera parviflora*; found in the Malaysian mangrove forests.

7.2 CONCLUSION OF THE RESULTS

7.2.1 Ecology

The three mangrove species studied showed variable responses to tidal flooding. The effects of prolonged tidal inundation in the greenhouse and in the field indicated stunted growth and decreased survival of *A. alba* seedlings under sea level rise. Similarly, tidal flooding reduced relative growth rates (RGR) and altered biomass allocations. The growth responses of *B. parviflora* were similar to that of *A. alba*, where tidal flooding decreased growth and altered biomass allocations. In contrast, tidal treatments had little effect on growth and biomass partitioning of *A. marina*.

Deep treatment decreased the soil redox potential and pH which impose a number of stresses to the growing seedling. Soil reduction was more intense in the deep treatment of

A. alba and *B. parviflora* suggesting that aeration of immediate rhizosphere in these species was less compared to *A. marina*.

7.2.2 Physiology

The physiological responses of *A. alba* indicated reduced assimilation rate and stomatal conductance under tidal flooding. Similarly, carbon sequestration was highly reduced during the first three months but the differences in these parameters were less variable during the sixth month which indicated that these responses were time dependent. Similar responses were observed between stomatal conductance, transpiration and atmospheric carbon dioxide concentrations.

The physiological responses of *A. marina* and *B. parviflora* under tidal treatments further supported the findings of the growth responses. The physiological parameters of *A. marina* were affected to a lesser extent than that of *B. parviflora* under prolonged tidal inundation. Although, carbon sequestration of *A. marina* and *B. parviflora* were reduced in the deep treatment, the reduction was much prominent in *B. parviflora* in the deep treatment. Apart from reduced growth and assimilation. Correlation of the ecological and physiological parameters indicated significant positive correlations between these parameters. Similarly, soil pH and Eh had significant positive effects on physiological parameters.

7.3 CONTRIBUTION OF THE PROJECT

The current research provides evidence on the differential tolerance of the three mangrove species studied. To date, there are no similar studies on *A. alba* and *B. parviflora*, two dominant species in the Malaysian mangrove forests. It is important to evaluate the

responses of mangrove species to ongoing climate change components for successful rehabilitation and restoration of mangrove forests in the context of the current climate change. Thus, findings presented in this research could be utilized in selecting mangrove species that best survive at different elevations which could pay off the efforts laid for successful rehabilitation of degraded mangrove forests.

7.4 CHALLENGES AND CONFLICTS OF INTEREST

The current research focuses on the growth and survival of mangrove seedlings under two tidal treatments. Further analyses of growth and physiological responses at various elevations and using a number of tidal treatments could provide better understanding on the effects of tidal inundation on these parameters. Similarly, long term exposure to both tidal treatments and different carbon dioxide concentrations will provide a better picture of the ecological and physiological responses of mangrove plants in the future under climate change. Likewise, it is anticipated that the responses of seedlings may differ from saplings suggesting that the responses are age dependent. Hence further evaluations using seedlings and saplings could provide insight into this phenomenon. Nevertheless, much effort is laid in this study to understand how elevated sea level might affect seedlings of the three species studied.

7.5 CONCLUSION

Tidal inundation induced stunted growth in *A. alba* and *B. parviflora* seedlings. Similarly, tidal flooding altered biomass allocations in these two species. In contrast, tidal treatments had little effects on the growth and biomass allocations of *A. marina*. Similarly, the

physiological responses of *A. alba* and *B. parviflora* varied significantly between tidal treatments whereas the physiological responses of *A. marina* was less sensitive to tidal flooding.

The observed growth and physiological responses could explain the distribution of these three species along the intertidal zones. Both *A. alba* and *B. parviflora* occupy the upper intertidal zones and land ward margins of the mangrove forests. In contrast, *A. marina* predominate the lower intertidal zones where salinity and tidal inundation is relatively higher than that of upper intertidal zones. In light of these results, it is concluded that both *A. alba* and *B. parviflora* are less tolerant to sea level rise while *A. marina* is better adapted to cope with the stresses of rising sea levels. These findings could aid mangrove rehabilitation and restoration in the context of a changing environment.

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